

100-101122 PCT/10-30 OCT 2001

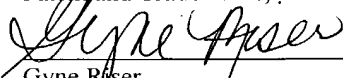
FORM PCT-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		ATTORNEY DOCKET NUMBER CHIR-0319
		U.S. APPLICATION NO. (if known see 37 C.F.R. 1.5) <div style="font-size: 1.5em; font-weight: bold;">10/018470</div>
INTERNATIONAL APPLICATION NO. PCT/US00/05928	INTERNATIONAL FILING DATE 08 March 2000	PRIORITY DATE CLAIMED 30 April 1999
TITLE OF INVENTION NEISSERIA GENOMIC SEQUENCES AND METHODS OF THEIR USE		
APPLICANT(S) FOR DO/EO/US Mariagrazia PIZZA, Erin HICKEY, Jeremy PETERSON, Herve TETTELIN, Craig J. VENTER, Vega MASIGNANI, Cesira GALEOTTI, Marirosa MORA, Giulio RATTI, Maria SCARSELLI, Vincenzo SCARLATO, Rino RAPPUOLI, Claire M. FRAZER and Guido GRANDI		
<p>Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:</p> <ol style="list-style-type: none"> 1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. 2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. 3. <input checked="" type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1). 4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date. 5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)). <ol style="list-style-type: none"> a. <input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau). b. <input type="checkbox"/> has been transmitted by the International Bureau. c. <input checked="" type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US) 6. <input type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)). 7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) <ol style="list-style-type: none"> a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau). b. <input type="checkbox"/> have been transmitted by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input checked="" type="checkbox"/> have not been made and will not be made. 8. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)). 9. <input type="checkbox"/> An oath or declaration of the inventor(s) 35 U.S.C. 371(c)(4). 10. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)). <p>Items 11. to 16. below concern other document(s) or information included:</p> <ol style="list-style-type: none"> 11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98. 12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. 13. <input type="checkbox"/> A FIRST preliminary amendment. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment. 14. <input type="checkbox"/> A substitute specification. 15. <input type="checkbox"/> A change of power of attorney and/or address letter. 16. <input checked="" type="checkbox"/> Other items or information: <ul style="list-style-type: none"> - A copy of the Published PCT Application by WIPO under No. WO 00/66791, including the search report. - A copy of the International Preliminary Examination Report. - Sequence listing in written (1,254 sheets) and computer readable form (3 diskettes) and Statement to Support Filing and Submission. 		
EXPRESS MAIL Mailing Label No. EL 922205442 US Date of Deposit: 30 October 2001 I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231 MAILER <u>John Hill</u> SIGNATURE <u><i>John Hill</i></u>		
<div style="font-size: 1.2em; font-weight: bold;">EL 922205442US</div>		

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581 Rec'd PC 30 OCT 2001

U.S. APPLICATION NO. (if known) 10/018470 INTERNATIONAL APPLICATION NO. PCT/US00/05928		ATTORNEY DOCKET NUMBER CHIR-0319		
17. <u> </u> The following fees are submitted: Basic National Fee (37 CFR 1.492(a)(1) - (5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO..... \$1,040.00 International preliminary examination fee (37 CFR 1.482 not paid to USPTO but International Search Report has been prepared by the EPO or JPO..... \$890.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO..... \$740.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)..... \$710.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)..... \$100.00 <div style="text-align: right;">ENTER APPROPRIATE BASIC FEE AMOUNT =</div>		<div style="border-bottom: 1px solid black; display: flex; justify-content: space-between; padding-bottom: 5px;"> CALCULATIONS PTO USE ONLY </div>		
Surcharge of \$130.00 for furnishing the oath or declaration later than <u> </u> 20 <u> </u> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).		<div style="border-bottom: 1px solid black; display: flex; justify-content: space-between; padding-bottom: 5px;"> \$ </div>		
Claims	Number Filed	Number Extra	Rate	
Total claims	- 20 =		X \$18.00	\$
Independent Claims	- 3 =		x \$84.00	\$
Multiple dependent claims(s) (if applicable)			+ \$280.00	\$
TOTAL OF ABOVE CALCULATIONS =				\$890.00
<u> </u> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				\$
SUBTOTAL =				\$890.00
Processing fee of \$130.00 for furnishing the English translation later than <u> </u> 20 <u> </u> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				+ \$
TOTAL NATIONAL FEE =				\$890.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property				+ \$
TOTAL FEES ENCLOSED =				\$890.00
				Amount to be:
				refunded
				\$
				charged
				\$
a. <input checked="" type="checkbox"/> A check in the amount of \$ 890.00 to cover the above fee is enclosed. b. <u> </u> Please charge my Deposit Account No. 23-3050 in the amount of \$ <u> </u> to cover the above fees. A duplicate copy of this sheet is enclosed. c. <u> </u> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 23-3050. A duplicate copy of this sheet is enclosed.				
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.				
SEND ALL CORRESPONDENCE TO:				
Alisa A. Harbin CHIRON CORPORATION Intellectual Property - R338 P.O. Box 8097 Emeryville, California 94662-8097		<div style="margin-bottom: 10px;"> SIGNATURE </div> <div style="margin-bottom: 10px;"> <u>Mark J. Rosen</u> NAME </div> <div> <u>39,822</u> REGISTRATION NUMBER </div>		

101 Rec'd PCT/PTO 21 NOV 2002
10/018470 PATENT
Atty. Dkt. No. CHIR-0319
Client Dkt. No. PP0365.322 #4

I hereby certify that this paper is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" on **November 21, 2002** and addressed to: BOX PCT, Assistant Commissioner of Patent and Trademarks, Washington, D.C. 20231.


Gyne Riser

11.21.02
Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of: **Pizza, M., et al.**

U.S. Serial No.: **10/018,470**

Int'l. Appln. No.: **PCT/US00/05928**

Group Art Unit: **not yet assigned**

Int. Filing Date: **March 8, 2000**

Examiner: **not yet assigned**

For: **NEISSERIA GENOMIC SEQUENCES AND METHODS OF THEIR USE**

Assistant Commissioner for Patents

Washington, D.C. 20231

BOX PCT

PRELIMINARY AMENDMENT

Applicants respectfully request entry of following amendments prior to examination on the merits, without prejudice.

In the Specification:

Please delete the Sequence Listing and insert therefore the new Sequence Listing.

In the Claims:

Please amend the claims to read as follows.

PATENT
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3. (Amended) A method for producing a protein, comprising the step of expressing a protein comprising an amino acid sequence identified according to claim 1.
5. (Amended) Nucleic acid comprising an open reading frame or protein-coding sequence identified by a method according to claim 1.
11. (Amended) Nucleic acid complementary to the nucleic acid of claim 7.
15. (Amended) Nucleic acid encoding a protein according to claim 6.
16. (Amended) A computer, a computer memory, a computer storage medium or a computer database containing the nucleotide sequence of a nucleic acid according to claim 8.
18. (Amended) A polyclonal or monoclonal antibody which binds to a protein according to claim 6.
19. (Amended) A nucleic acid probe comprising nucleic acid according to claim 5.
20. (Amended) An amplification primer comprising nucleic acid according to claim 5.
21. (Amended) A composition comprising nucleic acid according to claim 5.

Please cancel claims 22 and 23.

Please add the following new claims:

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25. (New) A method for producing a protein, comprising the step of expressing a protein comprising an amino acid sequence identified according to claim 2.
26. (New) A method for identifying a protein in *N. meningitidis*, comprising the steps of producing a protein according to claim 25, producing an antibody which binds to the protein, and determining whether the antibody recognizes a protein produced by *N. meningitidis*.
27. (New) Nucleic acid comprising an open reading frame or protein-coding sequence identified by a method according to claim 2.
28. (New) A protein obtained by the method of claim 25.
29. (New) Nucleic acid encoding a protein according to claim 28.
30. (New) A computer, a computer memory, a computer storage medium or a computer database containing the nucleotide sequence of a nucleic acid according to claim 9.
31. (New) A computer, a computer memory, a computer storage medium or a computer database containing the nucleotide sequence of a nucleic acid according to claim 10.
32. (New) A computer, a computer memory, a computer storage medium or a computer database containing the nucleotide sequence of a nucleic acid according to claim 11.
33. (New) A polyclonal or monoclonal antibody which binds to a protein according to claim 28.

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34. (New) A polyclonal or monoclonal antibody which binds to a protein according to claim 12.
35. (New) A polyclonal or monoclonal antibody which binds to a protein according to claim 13.
36. (New) A polyclonal or monoclonal antibody which binds to a protein according to claim 14.
37. (New) A nucleic acid probe comprising nucleic acid according to claim 27.
38. (New) A nucleic acid probe comprising nucleic acid according to claim 7.
39. (New) A nucleic acid probe comprising nucleic acid according to claim 8.
40. (New) A nucleic acid probe comprising nucleic acid according to claim 9.
41. (New) A nucleic acid probe comprising nucleic acid according to claim 10.
42. (New) A nucleic acid probe comprising nucleic acid according to claim 15.
43. (New) A nucleic acid probe comprising nucleic acid according to claim 29.
44. (New) An amplification primer comprising nucleic acid according to claim 27.
45. (New) An amplification primer comprising nucleic acid according to claim 7.
46. (New) An amplification primer comprising nucleic acid according to claim 8.

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47. (New) An amplification primer comprising nucleic acid according to claim 9.
48. (New) An amplification primer comprising nucleic acid according to claim 10.
49. (New) An amplification primer comprising nucleic acid according to claim 15.
50. (New) An amplification primer comprising nucleic acid according to claim 29.
51. (New) A composition comprising nucleic acid according to claim 27.
52. (New) A composition comprising nucleic acid according to claim 7.
53. (New) A composition comprising nucleic acid according to claim 8.
54. (New) A composition comprising nucleic acid according to claim 9.
55. (New) A composition comprising nucleic acid according to claim 10.
56. (New) A composition comprising nucleic acid according to claim 15.
57. (New) A composition comprising nucleic acid according to claim 29.
58. (New) A composition comprising protein according to claim 12.
59. (New) A composition comprising protein according to claim 13.
60. (New) A composition comprising protein according to claim 14.
61. (New) A composition comprising an antibody according to claim 18.

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- 62. (New) A composition comprising an antibody according to claim 33.
- 63. (New) A composition comprising an antibody according to claim 34.
- 64. (New) A composition comprising an antibody according to claim 35.
- 65. (New) A composition comprising an antibody according to claim 36.
- 66. (New) A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 51.
- 67. (New) A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 52.
- 68. (New) A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 53.
- 69. (New) A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 54.
- 70. (New) A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 55.
- 71. (New) A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 56.
- 72. (New) A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 57.

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73. (New) A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 58.
74. (New) A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 59.
75. (New) A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 60.
76. (New) A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 61.
77. (New) A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 62.
78. (New) A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 63.
79. (New) A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 64.
80. (New) A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 65.
81. (New) A method for detecting *N. meningitidis* antigens comprising contacting an antibody according to claim 18, 33, 34, 35, or 36 with a biological sample under conditions suitable for the formation of complexes between said antigens and said antibodies, and detecting said complexes.

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82. (New) A method for detecting antibodies that selectively bind to *N. meningitidis* antigens comprising contacting a protein according to claim 6, 12, 13, 14, or 28 with a biological sample under conditions suitable for the formation of complexes between said protein and said antibodies, and detecting said complexes.

REMARKS

Applicants submit the present amendment to correct improper multiple dependencies in the claims as originally filed. New claims 25 to 82 represent subject matter from original claims 1 to 24. Support for new claims 81 and 82 is found in the specification at, for example, page 10, lines 20 to 30. No new matter has been added.

The specification has been amended to delete the Sequence Listing that was originally filed with the application and to replace it with a new Sequence Listing. The new Sequence Listing is being submitted in response to objections raised to the original Sequence Listing in the Notification of Missing Requirements. No new matter has been added.

Applicants submit that the claims are in condition for allowance, and an early Office Action to that effect is earnestly solicited.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "Version with markings to show changes made."

Dated: 11/21/02

CHIRON CORPORATION
Intellectual Property - R440
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Respectfully submitted,

By: 

Alisa A. Harbin
Reg. No. 33,895

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Please delete the Sequence Listing and insert therefore the new Sequence Listing. I

In the Claims:

Please amend the claims as follows.

3. (Amended) A method for producing a protein, comprising the step of expressing a protein comprising an amino acid sequence identified according to [anyone of claims 1-2] claim 1.

5. (Amended) Nucleic acid comprising an open reading frame or protein-coding sequence identified by a method according to [anyone of claims 1-2] claim 1.

11. (Amended) Nucleic acid complementary to the nucleic acid of [anyone of claims 7-10] claim 7.

15. (Amended) Nucleic acid encoding a protein according to [anyone of claims 6- 8] claim 6.

16. (Amended) A computer, a computer memory, a computer storage medium or a computer database containing the nucleotide sequence of a nucleic acid according to [anyone of claims 7-11] claim 8.

18. (Amended) A polyclonal or monoclonal antibody which binds to a protein according to [anyone of claims 12-14 or 6] claim 6.

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19. (Amended) A nucleic acid probe comprising nucleic acid according to [any one of claims 5, 7-10, or 15] claim 5.

20. (Amended) An amplification primer comprising nucleic acid according to [any one of claims 5,7-10, or 15] claim 5.

21. Amended) A composition comprising [(a)] nucleic acid according [to anyone of claims 5, 7-10, or 15; (b) protein according to anyone of claims 12-14; and/or (c) an antibody according to claim 18] claim 5.

Claims 22 and 23 have been cancelled.

New claims 25 to 82 have been added.

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NEISSERIA GENOMIC SEQUENCES AND METHODS OF THEIR USE

This application claims priority to provisional U.S. application serial no. 60/132,068, filed 30 April 1999; PCT/US99/23573, filed 8 October 1999 (to be published April 2000); and Great Britain application serial no. GB-0004695.3, filed 28 February 2000.

This invention relates to methods of obtaining antigens and immunogens, the antigens and immunogens so obtained, and nucleic acids from the bacterial species: *Neisseria meningitidis*. In particular, it relates to genomic sequences from the bacterium; more particularly its "B" serogroup.

BACKGROUND

Neisseria meningitidis is a non-motile, gram negative diplococcus human pathogen. It colonizes the pharynx, causing meningitis and, occasionally, septicaemia in the absence of meningitis. It is closely related to *N. gonorrhoea*, although one feature that clearly differentiates meningococcus from gonococcus is the presence of a polysaccharide capsule that is present in all pathogenic meningococci.

N. meningitidis causes both endemic and epidemic disease. In the United States the attack rate is 0.6-1 per 100,000 persons per year, and it can be much greater during outbreaks. (see Lieberman *et al.* (1996) Safety and Immunogenicity of a Serogroups A/C *Neisseria meningitidis* Oligosaccharide-Protein Conjugate Vaccine in Young Children. *JAMA* 275(19):1499-1503; Schuchat *et al* (1997) Bacterial Meningitis in the United States in 1995. *N Engl J Med* 337(14):970-976). In developing countries, endemic disease rates are much higher and during epidemics incidence rates can reach 500 cases per 100,000 persons per year. Mortality is extremely high, at 10-20% in the United States, and much higher in developing countries. Following the introduction of the conjugate vaccine against *Haemophilus influenzae*, *N. meningitidis* is the major cause of bacterial meningitis at all ages in the United States (Schuchat *et al* (1997) *supra*).

Based on the organism's capsular polysaccharide, 12 serogroups of *N. meningitidis* have been identified. Group A is the pathogen most often implicated in epidemic disease in sub-Saharan Africa. Serogroups B and C are responsible for the vast majority of cases in the

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United States and in most developed countries. Serogroups W135 and Y are responsible for the rest of the cases in the United States and developed countries. The meningococcal vaccine currently in use is a tetravalent polysaccharide vaccine composed of serogroups A, C, Y and W135. Although efficacious in adolescents and adults, it induces a poor immune response and short duration of protection, and cannot be used in infants (e.g., Morbidity and Mortality weekly report, Vol. 46, No. RR-5 (1997)). This is because polysaccharides are T-cell independent antigens that induce a weak immune response that cannot be boosted by repeated immunization. Following the success of the vaccination against *H. influenzae*, conjugate vaccines against serogroups A and C have been developed and are at the final stage of clinical testing (Zollinger WD "New and Improved Vaccines Against Meningococcal Disease". In: *New Generation Vaccines, supra*, pp. 469-488; Lieberman *et al* (1996) *supra*; Costantino *et al* (1992) Development and phase I clinical testing of a conjugate vaccine against meningococcus A (menA) and C (menC) (*Vaccine* 10:691-698)).

Meningococcus B (MenB) remains a problem, however. This serotype currently is responsible for approximately 50% of total meningitis in the United States, Europe, and South America. The polysaccharide approach cannot be used because the MenB capsular polysaccharide is a polymer of $\alpha(2-8)$ -linked *N*-acetyl neuraminic acid that is also present in mammalian tissue. This results in tolerance to the antigen; indeed, if an immune response were elicited, it would be anti-self, and therefore undesirable. In order to avoid induction of autoimmunity and to induce a protective immune response, the capsular polysaccharide has, for instance, been chemically modified substituting the *N*-acetyl groups with *N*-propionyl groups, leaving the specific antigenicity unaltered (Romero & Outschoorn (1994) Current status of Meningococcal group B vaccine candidates: capsular or non-capsular? *Clin Microbiol Rev* 7(4):559-575).

Alternative approaches to MenB vaccines have used complex mixtures of outer membrane proteins (OMPs), containing either the OMPs alone, or OMPs enriched in porins, or deleted of the class 4 OMPs that are believed to induce antibodies that block bactericidal activity. This approach produces vaccines that are not well characterized. They are able to protect against the homologous strain, but are not effective at large where there are many antigenic variants of the outer membrane proteins. To overcome the antigenic variability, multivalent vaccines containing up to nine different porins have been constructed (e.g.,

Poolman JT (1992) Development of a meningococcal vaccine. *Infect. Agents Dis.* 4:13-28). Additional proteins to be used in outer membrane vaccines have been the opa and opc proteins, but none of these approaches have been able to overcome the antigenic variability (e.g., Ala'Aldeen & Borriello (1996) The meningococcal transferrin-binding proteins 1 and 2 are both surface exposed and generate bactericidal antibodies capable of killing homologous and heterologous strains. *Vaccine* 14(1):49-53).

A certain amount of sequence data is available for meningococcal and gonococcal genes and proteins (e.g., EP-A-0467714, WO96/29412), but this is by no means complete. The provision of further sequences could provide an opportunity to identify secreted or surface-exposed proteins that are presumed targets for the immune system and which are not antigenically variable or at least are more antigenically conserved than other and more variable regions. Thus, those antigenic sequences that are more highly conserved are preferred sequences. Those sequences specific to *Neisseria meningitidis* or *Neisseria gonorrhoeae* that are more highly conserved are further preferred sequences. For instance, some of the identified proteins could be components of efficacious vaccines against meningococcus B, some could be components of vaccines against all meningococcal serotypes, and others could be components of vaccines against all pathogenic *Neisseriae*. The identification of sequences from the bacterium will also facilitate the production of biological probes, particularly organism-specific probes.

It is thus an object of the invention is to provide Neisserial DNA sequences which (1) encode proteins predicted and/or shown to be antigenic or immunogenic, (2) can be used as probes or amplification primers, and (3) can be analyzed by bioinformatics.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates the products of protein expression and purification of the predicted ORF 919 as cloned and expressed in *E. coli*.

Fig. 2 illustrates the products of protein expression and purification of the predicted ORF 279 as cloned and expressed in *E. coli*.

Fig. 3 illustrates the products of protein expression and purification of the predicted ORF 576-1 as cloned and expressed in *E. coli*.



Fig. 4 illustrates the products of protein expression and purification of the predicted ORF 519-1 as cloned and expressed in *E. coli*.

Fig. 5 illustrates the products of protein expression and purification of the predicted ORF 121-1 as cloned and expressed in *E. coli*.

Fig. 6 illustrates the products of protein expression and purification of the predicted ORF 128-1 as cloned and expressed in *E. coli*.

Fig. 7 illustrates the products of protein expression and purification of the predicted ORF 206 as cloned and expressed in *E. coli*.

Fig. 8 illustrates the products of protein expression and purification of the predicted ORF 287 as cloned and expressed in *E. coli*.

Fig. 9 illustrates the products of protein expression and purification of the predicted ORF 406 as cloned and expressed in *E. coli*.

Fig. 10 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 919 as cloned and expressed in *E. coli*.

Fig. 11 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 279 as cloned and expressed in *E. coli*.

Fig. 12 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 576-1 as cloned and expressed in *E. coli*.

Fig. 13 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 519-1 as cloned and expressed in *E. coli*.

Fig. 14 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 121-1 as cloned and expressed in *E. coli*.

Fig. 15 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 128-1 as cloned and expressed in *E. coli*.

Fig. 16 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 206 as cloned and expressed in *E. coli*.

Fig. 17 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 287 as cloned and expressed in *E. coli*.

Fig. 18 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 406 as cloned and expressed in *E. coli*.

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THE INVENTION

The first complete sequence of the genome of *N. meningitidis* was disclosed as 961 partial contiguous nucleotide sequences, shown as SEQ ID NOs:1-961 of co-owned PCT/US99/23573 (the '573 application), filed 8 October 1999 (to be published April 2000). A single sequence full length genome of *N. meningitidis* was also disclosed as SEQ ID NO. 1068 of the '573 application. The invention is based on a full length genome of *N. meningitidis* which appears as SEQ ID NO. 1 in the present application as Appendix A hereto. The 961 sequences of the '573 application represent substantially the whole genome of serotype B of *N. meningitidis* (>99.98%). There is partial overlap between some of the 961 contiguous sequences ("contigs") shown in the 961 sequences, which overlap was used to construct the single full length sequence shown in SEQ ID NO. 1 in Appendix A hereto, using the TIGR Assembler [G.S. Sutton et al., *TIGR Assembler: A New Tool for Assembling Large Shotgun Sequencing Projects*, Genome Science and Technology, 1:9-19 (1995)]. Some of the nucleotides in the contigs had been previously released. (See ftp://ftp.tigr.org/pub/data/n_meningitidis on the world-wide web or "WWW"). The coordinates of the 2508 released sequences in the present contigs are presented in Appendix A of the '573 application. These data include the contig number (or i.d.) as presented in the first column; the name of the sequence as found on WWW is in the second column; with the coordinates of the contigs in the third and fourth columns, respectively. The sequences of certain MenB ORFs presented in Appendix B of the '573 application feature in International Patent Application filed by Chiron SpA on October 9, 1998 (PCT/IB98/01665) and January 14, 1999 (PCT/IB99/00103) respectively. Appendix B hereto provides a listing of 2158 open reading frames contained within the full length sequence found in SEQ ID NO. 1 in Appendix A hereto. The information set forth in Appendix B hereto includes the "NMB" name of the sequence, the putative translation product, and the beginning and ending nucleotide positions within SEQ ID NO. 1 which comprise the open reading frames. These open reading frames are referred to herein as the "NMB open reading frames".

In a first aspect, the invention provides nucleic acid including the *N. meningitidis* nucleotide sequence shown in SEQ ID NO. 1 in Appendix A hereto. It also provides nucleic acid comprising sequences having sequence identity to the nucleotide sequence disclosed herein. Depending on the particular sequence, the degree of sequence identity is preferably

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greater than 50% (e.g., 60%, 70%, 80%, 90%, 95%, 99% or more). These sequences include, for instance, mutants and allelic variants. The degree of sequence identity cited herein is determined across the length of the sequence determined by the Smith-Waterman homology search algorithm as implemented in MPSRCH program (Oxford Molecular) using an affine gap search with the following parameters: gap open penalty 12, gap extension penalty 1.

The invention also provides nucleic acid including a fragment of one or more of the nucleotide sequences set out herein, including the NMB open reading frames shown in Appendix B hereto. The fragment should comprise at least n consecutive nucleotides from the sequences and, depending on the particular sequence, n is 10 or more (e.g., 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 60, 75, 100 or more). Preferably, the fragment is unique to the genome of *N. meningitidis*, that is to say it is not present in the genome of another organism. More preferably, the fragment is unique to the genome of strain B of *N. meningitidis*. The invention also provides nucleic acid that hybridizes to those provided herein. Conditions for hybridizing are disclosed herein.

The invention also provides nucleic acid including sequences complementary to those described above (e.g., for antisense, for probes, or for amplification primers).

Nucleic acid according to the invention can, of course, be prepared in many ways (e.g., by chemical synthesis, from DNA libraries, from the organism itself, etc.) and can take various forms (e.g., single-stranded, double-stranded, vectors, probes, primers, etc.). The term "nucleic acid" includes DNA and RNA, and also their analogs, such as those containing modified backbones, and also peptide nucleic acid (PNA) etc.

It will be appreciated that, as SEQ ID NOs:1-961 of the '573 application represent the substantially complete genome of the organism, with partial overlap, references to SEQ ID NOs:1-961 of the '573 application include within their scope references to the complete genomic sequence, that is, SEQ ID NO. 1 hereof. For example, where two SEQ ID NOs overlap, the invention encompasses the single sequence which is formed by assembling the two overlapping sequences, which full sequence will be found in SEQ ID NO. 1 hereof. Thus, for instance, a nucleotide sequence which bridges two SEQ ID NOs but is not present in its entirety in either SEQ ID NO is still within the scope of the invention. Such a sequence will be present in its entirety in the single full length sequence of SEQ ID NO. 1 of the present application.

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The invention also provides vectors including nucleotide sequences of the invention (e.g., expression vectors, sequencing vectors, cloning vectors, etc.) and host cells transformed with such vectors.

According to a further aspect, the invention provides a protein including an amino acid sequence encoded within a *N. meningitidis* nucleotide sequence set out herein. It also provides proteins comprising sequences having sequence identity to those proteins. Depending on the particular sequence, the degree of sequence identity is preferably greater than 50% (e.g., 60%, 70%, 80%, 90%, 95%, 99% or more). Sequence identity is determined as above disclosed. These homologous proteins include mutants and allelic variants, encoded within the *N. meningitidis* nucleotide sequence set out herein.

The invention further provides proteins including fragments of an amino acid sequence encoded within a *N. meningitidis* nucleotide sequence set out in the sequence listing. The fragments should comprise at least n consecutive amino acids from the sequences and, depending on the particular sequence, n is 7 or more (e.g., 8, 10, 12, 14, 16, 18, 20 or more). Preferably the fragments comprise an epitope from the sequence.

The proteins of the invention can, of course, be prepared by various means (e.g., recombinant expression, purification from cell culture, chemical synthesis, etc.) and in various forms (e.g. native, fusions etc.). They are preferably prepared in substantially isolated form (i.e., substantially free from other *N. meningitidis* host cell proteins).

Various tests can be used to assess the *in vivo* immunogenicity of the proteins of the invention. For example, the proteins can be expressed recombinantly or chemically synthesized and used to screen patient sera by immunoblot. A positive reaction between the protein and patient serum indicates that the patient has previously mounted an immune response to the protein in question; i.e., the protein is an immunogen. This method can also be used to identify immunodominant proteins.

The invention also provides nucleic acid encoding a protein of the invention.

In a further aspect, the invention provides a computer, a computer memory, a computer storage medium (e.g., floppy disk, fixed disk, CD-ROM, etc.), and/or a computer database containing the nucleotide sequence of nucleic acid according to the invention. Preferably, it contains one or more of the *N. meningitidis* nucleotide sequences set out herein.

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This may be used in the analysis of the *N. meningitidis* nucleotide sequences set out herein. For instance, it may be used in a search to identify open reading frames (ORFs) or coding sequences within the sequences.

In a further aspect, the invention provides a method for identifying an amino acid sequence, comprising the step of searching for putative open reading frames or protein-coding sequences within a *N. meningitidis* nucleotide sequence set out herein. Similarly, the invention provides the use of a *N. meningitidis* nucleotide sequence set out herein in a search for putative open reading frames or protein-coding sequences.

Open-reading frame or protein-coding sequence analysis is generally performed on a computer using standard bioinformatic techniques. Typical algorithms or program used in the analysis include ORFFINDER (NCBI), GENMARK [Borodovsky & McIninch (1993) *Computers Chem* 17:122-133], and GLIMMER [Salzberg et al. (1998) *Nucl Acids Res* 26:544-548].

A search for an open reading frame or protein-coding sequence may comprise the steps of searching a *N. meningitidis* nucleotide sequence set out herein for an initiation codon and searching the upstream sequence for an in-frame termination codon. The intervening codons represent a putative protein-coding sequence. Typically, all six possible reading frames of a sequence will be searched.

An amino acid sequence identified in this way can be expressed using any suitable system to give a protein. This protein can be used to raise antibodies which recognize epitopes within the identified amino acid sequence. These antibodies can be used to screen *N. meningitidis* to detect the presence of a protein comprising the identified amino acid sequence.

Furthermore, once an ORF or protein-coding sequence is identified, the sequence can be compared with sequence databases. Sequence analysis tools can be found at NCBI (<http://www.ncbi.nlm.nih.gov>) e.g., the algorithms BLAST, BLAST2, BLASTn, BLASTp, tBLASTn, BLASTx, & tBLASTx [see also Altschul *et al.* (1997) Gapped BLAST and PSI-BLAST: new generation of protein database search programs. *Nucleic Acids Research* 25:2289-3402]. Suitable databases for comparison include the nonredundant GenBank, EMBL, DDBJ and PDB sequences, and the nonredundant GenBank CDS translations, PDB,

SwissProt, Spupdate and PIR sequences. This comparison may give an indication of the function of a protein.

Hydrophobic domains in an amino acid sequence can be predicted using algorithms such as those based on the statistical studies of Esposti *et al.* [Critical evaluation of the hydropathy of membrane proteins (1990) *Eur J Biochem* 190:207-219]. Hydrophobic domains represent potential transmembrane regions or hydrophobic leader sequences, which suggest that the proteins may be secreted or be surface-located. These properties are typically representative of good immunogens.

Similarly, transmembrane domains or leader sequences can be predicted using the PSORT algorithm (<http://www.psort.nibb.ac.jp>), and functional domains can be predicted using the MOTIFS program (GCG Wisconsin & PROSITE).

The invention also provides nucleic acid including an open reading frame or protein-coding sequence present in a *N. meningitidis* nucleotide sequence set out herein. Furthermore, the invention provides a protein including the amino acid sequence encoded by this open reading frame or protein-coding sequence.

According to a further aspect, the invention provides antibodies which bind to these proteins. These may be polyclonal or monoclonal and may be produced by any suitable means known to those skilled in the art.

The antibodies of the invention can be used in a variety of ways, e.g., for confirmation that a protein is expressed, or to confirm where a protein is expressed. Labeled antibody (e.g., fluorescent labeling for FACS) can be incubated with intact bacteria and the presence of label on the bacterial surface confirms the location of the protein, for instance.

According to a further aspect, the invention provides compositions including protein, antibody, and/or nucleic acid according to the invention. These compositions may be suitable as vaccines, as immunogenic compositions, or as diagnostic reagents.

The invention also provides nucleic acid, protein, or antibody according to the invention for use as medicaments (e.g., as vaccines) or as diagnostic reagents. It also provides the use of nucleic acid, protein, or antibody according to the invention in the manufacture of (i) a medicament for treating or preventing infection due to Neisserial bacteria (ii) a diagnostic reagent for detecting the presence of Neisserial bacteria or of antibodies raised against Neisserial bacteria. Said Neisserial bacteria may be any species or

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strain (such as *N. gonorrhoeae*) but are preferably *N. meningitidis*, especially strain A, strain B or strain C.

In still yet another aspect, the present invention provides for compositions including proteins, nucleic acid molecules, or antibodies. More preferable aspects of the present invention are drawn to immunogenic compositions of proteins. Further preferable aspects of the present invention contemplate pharmaceutical immunogenic compositions of proteins or vaccines and the use thereof in the manufacture of a medicament for the treatment or prevention of infection due to Neisserial bacteria, preferably infection of MenB.

The invention also provides a method of treating a patient, comprising administering to the patient a therapeutically effective amount of nucleic acid, protein, and/or antibody according to the invention.

According to further aspects, the invention provides various processes.

A process for producing proteins of the invention is provided, comprising the step of culturing a host cell according to the invention under conditions which induce protein expression. A process which may further include chemical synthesis of proteins and/or chemical synthesis (at least in part) of nucleotides.

A process for detecting polynucleotides of the invention is provided, comprising the steps of: (a) contacting a nucleic probe according to the invention with a biological sample under hybridizing conditions to form duplexes; and (b) detecting said duplexes.

A process for detecting proteins of the invention is provided, comprising the steps of: (a) contacting an antibody according to the invention with a biological sample under conditions suitable for the formation of an antibody-antigen complexes; and (b) detecting said complexes.

Another aspect of the present invention provides for a process for detecting antibodies that selectably bind to antigens or polypeptides or proteins specific to any species or strain of Neisserial bacteria and preferably to strains of *N. gonorrhoeae* but more preferably to strains of *N. meningitidis*, especially strain A, strain B or strain C, more preferably MenB, where the process comprises the steps of: (a) contacting antigen or polypeptide or protein according to the invention with a biological sample under conditions suitable for the formation of an antibody-antigen complexes; and (b) detecting said complexes.

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Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

Methodology - Summary of standard procedures and techniques.

General

This invention provides *Neisseria meningitidis* MenB nucleotide sequences, amino acid sequences encoded therein. With these disclosed sequences, nucleic acid probe assays and expression cassettes and vectors can be produced. The proteins can also be chemically synthesized. The expression vectors can be transformed into host cells to produce proteins. The purified or isolated polypeptides can be used to produce antibodies to detect MenB proteins. Also, the host cells or extracts can be utilized for biological assays to isolate agonists or antagonists. In addition, with these sequences one can search to identify open reading frames and identify amino acid sequences. The proteins may also be used in immunogenic compositions and as vaccine components.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature e.g., Sambrook *Molecular Cloning; A Laboratory Manual, Second Edition* (1989); *DNA Cloning, Volumes I and ii* (D.N Glover ed. 1985); *Oligonucleotide Synthesis* (M.J. Gait ed, 1984); *Nucleic Acid Hybridization* (B.D. Hames & S.J. Higgins eds. 1984); *Transcription and Translation* (B.D. Hames & S.J. Higgins eds. 1984); *Animal Cell Culture* (R.I. Freshney ed. 1986); *Immobilized Cells and Enzymes* (IRL Press, 1986); B. Perbal, *A Practical Guide to Molecular Cloning* (1984); the *Methods in Enzymology* series (Academic Press, Inc.), especially volumes 154 & 155; *Gene Transfer Vectors for Mammalian Cells* (J.H. Miller and M.P. Calos eds. 1987, Cold Spring Harbor Laboratory); Mayer and Walker, eds. (1987), *Immunochemical Methods in Cell and Molecular Biology* (Academic Press, London); Scopes, (1987) *Protein Purification: Principles and Practice*, Second Edition (Springer-Verlag, N.Y.), and *Handbook of Experimental Immunology, Volumes I-IV* (D.M. Weir and C.C. Blackwell eds 1986).

Standard abbreviations for nucleotides and amino acids are used in this specification.

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All publications, patents, and patent applications cited herein are incorporated in full by reference.

Expression systems

The *Neisseria* MenB nucleotide sequences can be expressed in a variety of different expression systems; for example those used with mammalian cells, plant cells, baculoviruses, bacteria, and yeast.

i. Mammalian Systems

Mammalian expression systems are known in the art. A mammalian promoter is any DNA sequence capable of binding mammalian RNA polymerase and initiating the downstream (3') transcription of a coding sequence (e.g., structural gene) into mRNA. A promoter will have a transcription initiating region, which is usually placed proximal to the 5' end of the coding sequence, and a TATA box, usually located 25-30 base pairs (bp) upstream of the transcription initiation site. The TATA box is thought to direct RNA polymerase II to begin RNA synthesis at the correct site. A mammalian promoter will also contain an upstream promoter element, usually located within 100 to 200 bp upstream of the TATA box. An upstream promoter element determines the rate at which transcription is initiated and can act in either orientation (Sambrook et al. (1989) "Expression of Cloned Genes in Mammalian Cells." In *Molecular Cloning: A Laboratory Manual*, 2nd ed.).

Mammalian viral genes are often highly expressed and have a broad host range; therefore sequences encoding mammalian viral genes provide particularly useful promoter sequences. Examples include the SV40 early promoter, mouse mammary tumor virus LTR promoter, adenovirus major late promoter (Ad MLP), and herpes simplex virus promoter. In addition, sequences derived from non-viral genes, such as the murine metallothionein gene, also provide useful promoter sequences. Expression may be either constitutive or regulated (inducible). Depending on the promoter selected, many promoters may be inducible using known substrates, such as the use of the mouse mammary tumor virus (MMTV) promoter with the glucocorticoid responsive element (GRE) that is induced by glucocorticoid in hormone-responsive transformed cells (see for example, U.S. Patent 5,783,681).

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The presence of an enhancer element (enhancer), combined with the promoter elements described above, will usually increase expression levels. An enhancer is a regulatory DNA sequence that can stimulate transcription up to 1000-fold when linked to homologous or heterologous promoters, with synthesis beginning at the normal RNA start site. Enhancers are also active when they are placed upstream or downstream from the transcription initiation site, in either normal or flipped orientation, or at a distance of more than 1000 nucleotides from the promoter (Maniatis et al. (1987) *Science* 236:1237; Alberts et al. (1989) *Molecular Biology of the Cell*, 2nd ed.). Enhancer elements derived from viruses may be particularly useful, because they usually have a broader host range. Examples include the SV40 early gene enhancer (Dijkema et al (1985) *EMBO J.* 4:761) and the enhancer/promoters derived from the long terminal repeat (LTR) of the Rous Sarcoma Virus (Gorman et al. (1982b) *Proc. Natl. Acad. Sci.* 79:6777) and from human cytomegalovirus (Boshart et al. (1985) *Cell* 41:521). Additionally, some enhancers are regulatable and become active only in the presence of an inducer, such as a hormone or metal ion (Sassone-Corsi and Borelli (1986) *Trends Genet.* 2:215; Maniatis et al. (1987) *Science* 236:1237).

A DNA molecule may be expressed intracellularly in mammalian cells. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus of the recombinant protein will always be a methionine, which is encoded by the ATG start codon. If desired, the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide.

Alternatively, foreign proteins can also be secreted from the cell into the growth media by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provides for secretion of the foreign protein in mammalian cells. Preferably, there are processing sites encoded between the leader fragment and the foreign gene that can be cleaved either *in vivo* or *in vitro*. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell. The adenovirus tripartite leader is an example of a leader sequence that provides for secretion of a foreign protein in mammalian cells.

Usually, transcription termination and polyadenylation sequences recognized by mammalian cells are regulatory regions located 3' to the translation stop codon and thus, together with the promoter elements, flank the coding sequence. The 3' terminus of the

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mature mRNA is formed by site-specific post-transcriptional cleavage and polyadenylation (Birnstiel et al. (1985) *Cell* 41:349; Proudfoot and Whitelaw (1988) "Termination and 3' end processing of eukaryotic RNA. In *Transcription and splicing* (ed. B.D. Hames and D.M. Glover); Proudfoot (1989) *Trends Biochem. Sci.* 14:105). These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Examples of transcription terminator/polyadenylation signals include those derived from SV40 (Sambrook et al (1989) "Expression of cloned genes in cultured mammalian cells." In *Molecular Cloning: A Laboratory Manual*).

Usually, the above-described components, comprising a promoter, polyadenylation signal, and transcription termination sequence are put together into expression constructs. Enhancers, introns with functional splice donor and acceptor sites, and leader sequences may also be included in an expression construct, if desired. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (e.g., plasmids) capable of stable maintenance in a host, such as mammalian cells or bacteria. Mammalian replication systems include those derived from animal viruses, which require trans-acting factors to replicate. For example, plasmids containing the replication systems of papovaviruses, such as SV40 (Gluzman (1981) *Cell* 23:175) or polyomavirus, replicate to extremely high copy number in the presence of the appropriate viral T antigen. Additional examples of mammalian replicons include those derived from bovine papillomavirus and Epstein-Barr virus. Additionally, the replicon may have two replication systems, thus allowing it to be maintained, for example, in mammalian cells for expression and in a prokaryotic host for cloning and amplification. Examples of such mammalian-bacteria shuttle vectors include pMT2 (Kaufman et al. (1989) *Mol. Cell. Biol.* 9:946) and pHEBO (Shimizu et al. (1986) *Mol. Cell. Biol.* 6:1074).

The transformation procedure used depends upon the host to be transformed. Methods for introduction of heterologous polynucleotides into mammalian cells are known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

Mammalian cell lines available as hosts for expression are known in the art and include many immortalized cell lines available from the American Type Culture Collection

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(ATCC), including but not limited to, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), and a number of other cell lines.

ii. Plant Cellular Expression Systems

There are many plant cell culture and whole plant genetic expression systems known in the art. Exemplary plant cellular genetic expression systems include those described in patents, such as: U.S. 5,693,506; US 5,659,122; and US 5,608,143. Additional examples of genetic expression in plant cell culture has been described by Zenk, *Phytochemistry* 30:3861-3863 (1991). Descriptions of plant protein signal peptides may be found in addition to the references described above in Vaulcombe et al., *Mol. Gen. Genet.* 209:33-40 (1987); Chandler et al., *Plant Molecular Biology* 3:407-418 (1984); Rogers, *J. Biol. Chem.* 260:3731-3738 (1985); Rothstein et al., *Gene* 55:353-356 (1987); Whittier et al., *Nucleic Acids Research* 15:2515-2535 (1987); Wirsal et al., *Molecular Microbiology* 3:3-14 (1989); Yu et al., *Gene* 122:247-253 (1992). A description of the regulation of plant gene expression by the phytohormone, gibberellic acid and secreted enzymes induced by gibberellic acid can be found in R.L. Jones and J. MacMillin, *Gibberellins*: in: *Advanced Plant Physiology*, Malcolm B. Wilkins, ed., 1984 Pitman Publishing Limited, London, pp. 21-52. References that describe other metabolically-regulated genes: Sheen, *Plant Cell*, 2:1027-1038(1990); Maas et al., *EMBO J.* 9:3447-3452 (1990); Benkel and Hickey, *Proc. Natl. Acad. Sci.* 84:1337-1339 (1987)

Typically, using techniques known in the art, a desired polynucleotide sequence is inserted into an expression cassette comprising genetic regulatory elements designed for operation in plants. The expression cassette is inserted into a desired expression vector with companion sequences upstream and downstream from the expression cassette suitable for expression in a plant host. The companion sequences will be of plasmid or viral origin and provide necessary characteristics to the vector to permit the vectors to move DNA from an original cloning host, such as bacteria, to the desired plant host. The basic bacterial/plant vector construct will preferably provide a broad host range prokaryote replication origin; a prokaryote selectable marker; and, for *Agrobacterium* transformations, T DNA sequences for *Agrobacterium*-mediated transfer to plant chromosomes. Where the heterologous gene is not

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readily amenable to detection, the construct will preferably also have a selectable marker gene suitable for determining if a plant cell has been transformed. A general review of suitable markers, for example for the members of the grass family, is found in Wilmink and Dons, 1993, *Plant Mol. Biol. Repr.*, 11(2):165-185.

Sequences suitable for permitting integration of the heterologous sequence into the plant genome are also recommended. These might include transposon sequences and the like for homologous recombination as well as Ti sequences which permit random insertion of a heterologous expression cassette into a plant genome. Suitable prokaryote selectable markers include resistance toward antibiotics such as ampicillin or tetracycline. Other DNA sequences encoding additional functions may also be present in the vector, as is known in the art.

The nucleic acid molecules of the subject invention may be included into an expression cassette for expression of the protein(s) of interest. Usually, there will be only one expression cassette, although two or more are feasible. The recombinant expression cassette will contain in addition to the heterologous protein encoding sequence the following elements, a promoter region, plant 5' untranslated sequences, initiation codon depending upon whether or not the structural gene comes equipped with one, and a transcription and translation termination sequence. Unique restriction enzyme sites at the 5' and 3' ends of the cassette allow for easy insertion into a pre-existing vector.

A heterologous coding sequence may be for any protein relating to the present invention. The sequence encoding the protein of interest will encode a signal peptide which allows processing and translocation of the protein, as appropriate, and will usually lack any sequence which might result in the binding of the desired protein of the invention to a membrane. Since, for the most part, the transcriptional initiation region will be for a gene which is expressed and translocated during germination, by employing the signal peptide which provides for translocation, one may also provide for translocation of the protein of interest. In this way, the protein(s) of interest will be translocated from the cells in which they are expressed and may be efficiently harvested. Typically secretion in seeds are across the aleurone or scutellar epithelium layer into the endosperm of the seed. While it is not required that the protein be secreted from the cells in which the protein is produced, this facilitates the isolation and purification of the recombinant protein.

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Since the ultimate expression of the desired gene product will be in a eucaryotic cell it is desirable to determine whether any portion of the cloned gene contains sequences which will be processed out as introns by the host's splicosome machinery. If so, site-directed mutagenesis of the "intron" region may be conducted to prevent losing a portion of the genetic message as a false intron code, Reed and Maniatis, *Cell* 41:95-105, 1985.

The vector can be microinjected directly into plant cells by use of micropipettes to mechanically transfer the recombinant DNA. Crossway, *Mol. Gen. Genet.*, 202:179-185, 1985. The genetic material may also be transferred into the plant cell by using polyethylene glycol, Krens, et al., *Nature*, 296, 72-74, 1982. Another method of introduction of nucleic acid segments is high velocity ballistic penetration by small particles with the nucleic acid either within the matrix of small beads or particles, or on the surface, Klein, et al., *Nature*, 327, 70-73, 1987 and Knudsen and Muller, 1991, *Planta*, 185:330-336 teaching particle bombardment of barley endosperm to create transgenic barley. Yet another method of introduction would be fusion of protoplasts with other entities, either minicells, cells, lysosomes or other fusible lipid-surfaced bodies, Fraley, et al., *Proc. Natl. Acad. Sci. USA*, 79, 1859-1863, 1982.

The vector may also be introduced into the plant cells by electroporation. (Fromm et al., *Proc. Natl. Acad. Sci. USA* 82:5824, 1985). In this technique, plant protoplasts are electroporated in the presence of plasmids containing the gene construct. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and form plant callus.

All plants from which protoplasts can be isolated and cultured to give whole regenerated plants can be transformed by the present invention so that whole plants are recovered which contain the transferred gene. It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to all major species of sugarcane, sugar beet, cotton, fruit and other trees, legumes and vegetables. Some suitable plants include, for example, species from the genera *Fragaria*, *Lotus*, *Medicago*, *Onobrychis*, *Trifolium*, *Trigonella*, *Vigna*, *Citrus*, *Linum*, *Geranium*, *Manihot*, *Daucus*, *Arabidopsis*, *Brassica*, *Raphanus*, *Sinapis*, *Atropa*, *Capsicum*, *Datura*, *Hyoscyamus*, *Lycopersion*, *Nicotiana*, *Solanum*, *Petunia*, *Digitalis*, *Majorana*, *Cichorium*, *Helianthus*, *Lactuca*, *Bromus*, *Asparagus*, *Antirrhinum*, *Hererocallis*, *Nemesia*, *Pelargonium*, *Panicum*, *Pennisetum*,

Ranunculus, *Senecio*, *Salpiglossis*, *Cucumis*, *Browaalia*, *Glycine*, *Lolium*, *Zea*, *Triticum*, *Sorghum*, and *Datura*.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts containing copies of the heterologous gene is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced from the protoplast suspension. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Shoots and roots normally develop simultaneously. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is fully reproducible and repeatable.

In some plant cell culture systems, the desired protein of the invention may be excreted or alternatively, the protein may be extracted from the whole plant. Where the desired protein of the invention is secreted into the medium, it may be collected. Alternatively, the embryos and embryoless-half seeds or other plant tissue may be mechanically disrupted to release any secreted protein between cells and tissues. The mixture may be suspended in a buffer solution to retrieve soluble proteins. Conventional protein isolation and purification methods will be then used to purify the recombinant protein. Parameters of time, temperature pH, oxygen, and volumes will be adjusted through routine methods to optimize expression and recovery of heterologous protein.

iii. Baculovirus Systems

The polynucleotide encoding the protein can also be inserted into a suitable insect expression vector, and is operably linked to the control elements within that vector. Vector construction employs techniques which are known in the art. Generally, the components of the expression system include a transfer vector, usually a bacterial plasmid, which contains both a fragment of the baculovirus genome, and a convenient restriction site for insertion of the heterologous gene or genes to be expressed; a wild type baculovirus with a sequence homologous to the baculovirus-specific fragment in the transfer vector (this allows for the

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homologous recombination of the heterologous gene in to the baculovirus genome); and appropriate insect host cells and growth media.

After inserting the DNA sequence encoding the protein into the transfer vector, the vector and the wild type viral genome are transfected into an insect host cell where the vector and viral genome are allowed to recombine. The packaged recombinant virus is expressed and recombinant plaques are identified and purified. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, *inter alia*, Invitrogen, San Diego CA ("MaxBac" kit). These techniques are generally known to those skilled in the art and fully described in Summers and Smith, *Texas Agricultural Experiment Station Bulletin No. 1555* (1987) (hereinafter "Summers and Smith").

Prior to inserting the DNA sequence encoding the protein into the baculovirus genome, the above described components, comprising a promoter, leader (if desired), coding sequence of interest, and transcription termination sequence, are usually assembled into an intermediate transplacement construct (transfer vector). This construct may contain a single gene and operably linked regulatory elements; multiple genes, each with its own set of operably linked regulatory elements; or multiple genes, regulated by the same set of regulatory elements. Intermediate transplacement constructs are often maintained in a replicon, such as an extrachromosomal element (e.g., plasmids) capable of stable maintenance in a host, such as a bacterium. The replicon will have a replication system, thus allowing it to be maintained in a suitable host for cloning and amplification.

Currently, the most commonly used transfer vector for introducing foreign genes into AcNPV is pAc373. Many other vectors, known to those of skill in the art, have also been designed. These include, for example, pVL985 (which alters the polyhedrin start codon from ATG to ATT, and which introduces a BamHI cloning site 32 basepairs downstream from the ATT; see Luckow and Summers, *Virology* (1989) 17:31.

The plasmid usually also contains the polyhedrin polyadenylation signal (Miller et al. (1988) *Ann. Rev. Microbiol.*, 42:177) and a prokaryotic ampicillin-resistance (*amp*) gene and origin of replication for selection and propagation in *E. coli*.

Baculovirus transfer vectors usually contain a baculovirus promoter. A baculovirus promoter is any DNA sequence capable of binding a baculovirus RNA polymerase and initiating the downstream (5' to 3') transcription of a coding sequence (e.g., structural gene)

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into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site and a transcription initiation site. A baculovirus transfer vector may also have a second domain called an enhancer, which, if present, is usually distal to the structural gene. Expression may be either regulated or constitutive.

Structural genes, abundantly transcribed at late times in a viral infection cycle, provide particularly useful promoter sequences. Examples include sequences derived from the gene encoding the viral polyhedron protein, Friesen et al., (1986) "The Regulation of Baculovirus Gene Expression," in: *The Molecular Biology of Baculoviruses* (ed. Walter Doerfler); EPO Publ. Nos. 127 839 and 155 476; and the gene encoding the p10 protein, Vlcek et al., (1988), *J. Gen. Virol.* 69:765.

DNA encoding suitable signal sequences can be derived from genes for secreted insect or baculovirus proteins, such as the baculovirus polyhedrin gene (Carbonell et al. (1988) *Gene*, 73:409). Alternatively, since the signals for mammalian cell posttranslational modifications (such as signal peptide cleavage, proteolytic cleavage, and phosphorylation) appear to be recognized by insect cells, and the signals required for secretion and nuclear accumulation also appear to be conserved between the invertebrate cells and vertebrate cells, leaders of non-insect origin, such as those derived from genes encoding human (alpha) α -interferon, Maeda et al., (1985), *Nature* 315:592; human gastrin-releasing peptide, Lebacqz-Verheyden et al., (1988), *Molec. Cell. Biol.* 8:3129; human IL-2, Smith et al., (1985) *Proc. Nat'l Acad. Sci. USA*, 82:8404; mouse IL-3, (Miyajima et al., (1987) *Gene* 58:273; and human glucocerebrosidase, Martin et al. (1988) *DNA*, 7:99, can also be used to provide for secretion in insects.

A recombinant polypeptide or polyprotein may be expressed intracellularly or, if it is expressed with the proper regulatory sequences, it can be secreted. Good intracellular expression of nonfused foreign proteins usually requires heterologous genes that ideally have a short leader sequence containing suitable translation initiation signals preceding an ATG start signal. If desired, methionine at the N-terminus may be cleaved from the mature protein by *in vitro* incubation with cyanogen bromide.

Alternatively, recombinant polyproteins or proteins which are not naturally secreted can be secreted from the insect cell by creating chimeric DNA molecules that encode a fusion

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protein comprised of a leader sequence fragment that provides for secretion of the foreign protein in insects. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the translocation of the protein into the endoplasmic reticulum.

After insertion of the DNA sequence and/or the gene encoding the expression product precursor of the protein, an insect cell host is co-transformed with the heterologous DNA of the transfer vector and the genomic DNA of wild type baculovirus -- usually by co-transfection. The promoter and transcription termination sequence of the construct will usually comprise a 2-5kb section of the baculovirus genome. Methods for introducing heterologous DNA into the desired site in the baculovirus virus are known in the art. (See Summers and Smith *supra*; Ju et al. (1987); Smith et al., *Mol. Cell. Biol.* (1983) 3:2156; and Luckow and Summers (1989)). For example, the insertion can be into a gene such as the polyhedrin gene, by homologous double crossover recombination; insertion can also be into a restriction enzyme site engineered into the desired baculovirus gene. Miller et al., (1989), *Bioessays* 4:91. The DNA sequence, when cloned in place of the polyhedrin gene in the expression vector, is flanked both 5' and 3' by polyhedrin-specific sequences and is positioned downstream of the polyhedrin promoter.

The newly formed baculovirus expression vector is subsequently packaged into an infectious recombinant baculovirus. Homologous recombination occurs at low frequency (between about 1% and about 5%); thus, the majority of the virus produced after cotransfection is still wild-type virus. Therefore, a method is necessary to identify recombinant viruses. An advantage of the expression system is a visual screen allowing recombinant viruses to be distinguished. The polyhedrin protein, which is produced by the native virus, is produced at very high levels in the nuclei of infected cells at late times after viral infection. Accumulated polyhedrin protein forms occlusion bodies that also contain embedded particles. These occlusion bodies, up to 15 μm in size, are highly refractile, giving them a bright shiny appearance that is readily visualized under the light microscope. Cells infected with recombinant viruses lack occlusion bodies. To distinguish recombinant virus from wild-type virus, the transfection supernatant is plaqued onto a monolayer of insect cells by techniques known to those skilled in the art. Namely, the plaques are screened under the light microscope for the presence (indicative of wild-type virus) or absence (indicative of

recombinant virus) of occlusion bodies. *Current Protocols in Microbiology* Vol. 2 (Ausubel et al. eds) at 16.8 (Supp. 10, 1990); Summers and Smith, *supra*; Miller et al. (1989).

Recombinant baculovirus expression vectors have been developed for infection into several insect cells. For example, recombinant baculoviruses have been developed for, *inter alia*: *Aedes aegypti*, *Autographa californica*, *Bombyx mori*, *Drosophila melanogaster*, *Spodoptera frugiperda*, and *Trichoplusia ni* (PCT Pub. No. WO 89/046699; Carbonell et al., (1985) *J. Virol.* 56:153; Wright (1986) *Nature* 321:718; Smith et al., (1983) *Mol. Cell. Biol.* 3:2156; and see generally, Fraser, et al. (1989) *In Vitro Cell. Dev. Biol.* 25:225).

Cells and cell culture media are commercially available for both direct and fusion expression of heterologous polypeptides in a baculovirus/expression system; cell culture technology is generally known to those skilled in the art. See, e.g., Summers and Smith *supra*.

The modified insect cells may then be grown in an appropriate nutrient medium, which allows for stable maintenance of the plasmid(s) present in the modified insect host. Where the expression product gene is under inducible control, the host may be grown to high density, and expression induced. Alternatively, where expression is constitutive, the product will be continuously expressed into the medium and the nutrient medium must be continuously circulated, while removing the product of interest and augmenting depleted nutrients. The product may be purified by such techniques as chromatography, e.g., HPLC, affinity chromatography, ion exchange chromatography, etc.; electrophoresis; density gradient centrifugation; solvent extraction, or the like. As appropriate, the product may be further purified, as required, so as to remove substantially any insect proteins which are also secreted in the medium or result from lysis of insect cells, so as to provide a product which is at least substantially free of host debris, e.g., proteins, lipids and polysaccharides.

In order to obtain protein expression, recombinant host cells derived from the transformants are incubated under conditions which allow expression of the recombinant protein encoding sequence. These conditions will vary, dependent upon the host cell selected. However, the conditions are readily ascertainable to those of ordinary skill in the art, based upon what is known in the art.

iv. Bacterial Systems

Bacterial expression techniques are known in the art. A bacterial promoter is any DNA sequence capable of binding bacterial RNA polymerase and initiating the downstream (3') transcription of a coding sequence (e.g. structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site and a transcription initiation site. A bacterial promoter may also have a second domain called an operator, that may overlap an adjacent RNA polymerase binding site at which RNA synthesis begins. The operator permits negative regulated (inducible) transcription, as a gene repressor protein may bind the operator and thereby inhibit transcription of a specific gene. Constitutive expression may occur in the absence of negative regulatory elements, such as the operator. In addition, positive regulation may be achieved by a gene activator protein binding sequence, which, if present is usually proximal (5') to the RNA polymerase binding sequence. An example of a gene activator protein is the catabolite activator protein (CAP), which helps initiate transcription of the lac operon in *Escherichia coli* (*E. coli*) (Raibaud *et al.* (1984) *Annu. Rev. Genet.* 18:173). Regulated expression may therefore be either positive or negative, thereby either enhancing or reducing transcription.

Sequences encoding metabolic pathway enzymes provide particularly useful promoter sequences. Examples include promoter sequences derived from sugar metabolizing enzymes, such as galactose, lactose (*lac*) (Chang *et al.* (1977) *Nature* 198:1056), and maltose. Additional examples include promoter sequences derived from biosynthetic enzymes such as tryptophan (*trp*) (Goeddel *et al.* (1980) *Nuc. Acids Res.* 8:4057; Yelverton *et al.* (1981) *Nucl. Acids Res.* 9:731; U.S. Patent 4,738,921; EPO Publ. Nos. 036 776 and 121 775). The beta-lactamase (*bla*) promoter system (Weissmann (1981) "The cloning of interferon and other mistakes." In *Interferon 3* (ed. I. Gresser)), bacteriophage lambda PL (Shimatake *et al.* (1981) *Nature* 292:128) and T5 (U.S. Patent 4,689,406) promoter systems also provide useful promoter sequences.

In addition, synthetic promoters which do not occur in nature also function as bacterial promoters. For example, transcription activation sequences of one bacterial or bacteriophage promoter may be joined with the operon sequences of another bacterial or bacteriophage promoter, creating a synthetic hybrid promoter (U.S. Patent 4,551,433). For

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example, the *tac* promoter is a hybrid *trp-lac* promoter comprised of both *trp* promoter and *lac* operon sequences that is regulated by the *lac* repressor (Amann *et al.* (1983) *Gene* 25:167; de Boer *et al.* (1983) *Proc. Natl. Acad. Sci.* 80:21). Furthermore, a bacterial promoter can include naturally occurring promoters of non-bacterial origin that have the ability to bind bacterial RNA polymerase and initiate transcription. A naturally occurring promoter of non-bacterial origin can also be coupled with a compatible RNA polymerase to produce high levels of expression of some genes in prokaryotes. The bacteriophage T7 RNA polymerase/promoter system is an example of a coupled promoter system (Studier *et al.* (1986) *J. Mol. Biol.* 189:113; Tabor *et al.* (1985) *Proc Natl. Acad. Sci.* 82:1074). In addition, a hybrid promoter can also be comprised of a bacteriophage promoter and an *E. coli* operator region (EPO Publ. No. 267 851).

In addition to a functioning promoter sequence, an efficient ribosome binding site is also useful for the expression of foreign genes in prokaryotes. In *E. coli*, the ribosome binding site is called the Shine-Dalgarno (SD) sequence and includes an initiation codon (ATG) and a sequence 3-9 nucleotides in length located 3-11 nucleotides upstream of the initiation codon (Shine *et al.* (1975) *Nature* 254:34). The SD sequence is thought to promote binding of mRNA to the ribosome by the pairing of bases between the SD sequence and the 3' end of *E. coli* 16S rRNA (Steitz *et al.* (1979) "Genetic signals and nucleotide sequences in messenger RNA." In *Biological Regulation and Development: Gene Expression* (ed. R.F. Goldberger)). To express eukaryotic genes and prokaryotic genes with weak ribosome-binding site, it is often necessary to optimize the distance between the SD sequence and the ATG of the eukaryotic gene (Sambrook *et al.* (1989) "Expression of cloned genes in *Escherichia coli*." In *Molecular Cloning: A Laboratory Manual*).

A DNA molecule may be expressed intracellularly. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus will always be a methionine, which is encoded by the ATG start codon. If desired, methionine at the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide or by either *in vivo* or *in vitro* incubation with a bacterial methionine N-terminal peptidase (EPO Publ. No. 219 237).

Fusion proteins provide an alternative to direct expression. Usually, a DNA sequence encoding the N-terminal portion of an endogenous bacterial protein, or other stable protein, is

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fused to the 5' end of heterologous coding sequences. Upon expression, this construct will provide a fusion of the two amino acid sequences. For example, the bacteriophage lambda cell gene can be linked at the 5' terminus of a foreign gene and expressed in bacteria. The resulting fusion protein preferably retains a site for a processing enzyme (factor Xa) to cleave the bacteriophage protein from the foreign gene (Nagai *et al.* (1984) *Nature* 309:810). Fusion proteins can also be made with sequences from the *lacZ* (Jia *et al.* (1987) *Gene* 60:197), *trpE* (Allen *et al.* (1987) *J. Biotechnol.* 5:93; Makoff *et al.* (1989) *J. Gen. Microbiol.* 135:11), and *Chey* (EPO Publ. No. 324 647) genes. The DNA sequence at the junction of the two amino acid sequences may or may not encode a cleavable site. Another example is a ubiquitin fusion protein. Such a fusion protein is made with the ubiquitin region that preferably retains a site for a processing enzyme (e.g. ubiquitin specific processing-protease) to cleave the ubiquitin from the foreign protein. Through this method, native foreign protein can be isolated (Miller *et al.* (1989) *Bio/Technology* 7:698).

Alternatively, foreign proteins can also be secreted from the cell by creating chimeric DNA molecules that encode a fusion protein comprised of a signal peptide sequence fragment that provides for secretion of the foreign protein in bacteria (U.S. Patent 4,336,336). The signal sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell. The protein is either secreted into the growth media (gram-positive bacteria) or into the periplasmic space, located between the inner and outer membrane of the cell (gram-negative bacteria). Preferably there are processing sites, which can be cleaved either *in vivo* or *in vitro* encoded between the signal peptide fragment and the foreign gene.

DNA encoding suitable signal sequences can be derived from genes for secreted bacterial proteins, such as the *E. coli* outer membrane protein gene (*ompA*) (Masui *et al.* (1983), in: *Experimental Manipulation of Gene Expression*; Ghayeb *et al.* (1984) *EMBO J.* 3:2437) and the *E. coli* alkaline phosphatase signal sequence (*phoA*) (Oka *et al.* (1985) *Proc. Natl. Acad. Sci.* 82:7212). As an additional example, the signal sequence of the alpha-amylase gene from various *Bacillus* strains can be used to secrete heterologous proteins from *B. subtilis* (Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA* 79:5582; EPO Publ. No. 244 042).

Usually, transcription termination sequences recognized by bacteria are regulatory regions located 3' to the translation stop codon, and thus together with the promoter flank the

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coding sequence. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Transcription termination sequences frequently include DNA sequences of about 50 nucleotides capable of forming stem loop structures that aid in terminating transcription. Examples include transcription termination sequences derived from genes with strong promoters, such as the *trp* gene in *E. coli* as well as other biosynthetic genes.

Usually, the above described components, comprising a promoter, signal sequence (if desired), coding sequence of interest, and transcription termination sequence, are put together into expression constructs. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (e.g., plasmids) capable of stable maintenance in a host, such as bacteria. The replicon will have a replication system, thus allowing it to be maintained in a prokaryotic host either for expression or for cloning and amplification. In addition, a replicon may be either a high or low copy number plasmid. A high copy number plasmid will generally have a copy number ranging from about 5 to about 200, and usually about 10 to about 150. A host containing a high copy number plasmid will preferably contain at least about 10, and more preferably at least about 20 plasmids. Either a high or low copy number vector may be selected, depending upon the effect of the vector and the foreign protein on the host.

Alternatively, the expression constructs can be integrated into the bacterial genome with an integrating vector. Integrating vectors usually contain at least one sequence homologous to the bacterial chromosome that allows the vector to integrate. Integrations appear to result from recombinations between homologous DNA in the vector and the bacterial chromosome. For example, integrating vectors constructed with DNA from various *Bacillus* strains integrate into the *Bacillus* chromosome (EPO Publ. No. 127 328). Integrating vectors may also be comprised of bacteriophage or transposon sequences.

Usually, extrachromosomal and integrating expression constructs may contain selectable markers to allow for the selection of bacterial strains that have been transformed. Selectable markers can be expressed in the bacterial host and may include genes which render bacteria resistant to drugs such as ampicillin, chloramphenicol, erythromycin, kanamycin (neomycin), and tetracycline (Davies *et al.* (1978) *Annu. Rev. Microbiol.* 32:469). Selectable

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markers may also include biosynthetic genes, such as those in the histidine, tryptophan, and leucine biosynthetic pathways.

Alternatively, some of the above described components can be put together in transformation vectors. Transformation vectors are usually comprised of a selectable marker that is either maintained in a replicon or developed into an integrating vector, as described above.

Expression and transformation vectors, either extra-chromosomal replicons or integrating vectors, have been developed for transformation into many bacteria. For example, expression vectors have been developed for, *inter alia*, the following bacteria: *Bacillus subtilis* (Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA* 79:5582; EPO Publ. Nos. 036 259 and 063 953; PCT Publ. No. WO 84/04541), *Escherichia coli* (Shimatake *et al.* (1981) *Nature* 292:128; Amann *et al.* (1985) *Gene* 40:183; Studier *et al.* (1986) *J. Mol. Biol.* 189:113; EPO Publ. Nos. 036 776, 136 829 and 136 907), *Streptococcus cremoris* (Powell *et al.* (1988) *Appl. Environ. Microbiol.* 54:655); *Streptococcus lividans* (Powell *et al.* (1988) *Appl. Environ. Microbiol.* 54:655), *Streptomyces lividans* (U.S. Patent 4,745,056).

Methods of introducing exogenous DNA into bacterial hosts are well-known in the art, and usually include either the transformation of bacteria treated with CaCl₂ or other agents, such as divalent cations and DMSO. DNA can also be introduced into bacterial cells by electroporation. Transformation procedures usually vary with the bacterial species to be transformed. (See e.g., use of *Bacillus*: Masson *et al.* (1989) *FEMS Microbiol. Lett.* 60:273; Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA* 79:5582; EPO Publ. Nos. 036 259 and 063 953; PCT Publ. No. WO 84/04541; use of *Campylobacter*: Miller *et al.* (1988) *Proc. Natl. Acad. Sci.* 85:856; and Wang *et al.* (1990) *J. Bacteriol.* 172:949; use of *Escherichia coli*: Cohen *et al.* (1973) *Proc. Natl. Acad. Sci.* 69:2110; Dower *et al.* (1988) *Nucleic Acids Res.* 16:6127; Kushner (1978) "An improved method for transformation of *Escherichia coli* with ColE1-derived plasmids. In *Genetic Engineering: Proceedings of the International Symposium on Genetic Engineering* (eds. H.W. Boyer and S. Nicosia); Mandel *et al.* (1970) *J. Mol. Biol.* 53:159; Taketo (1988) *Biochim. Biophys. Acta* 949:318; use of *Lactobacillus*: Chassy *et al.* (1987) *FEMS Microbiol. Lett.* 44:173; use of *Pseudomonas*: Fiedler *et al.* (1988) *Anal. Biochem.* 170:38; use of *Staphylococcus*: Augustin *et al.* (1990) *FEMS Microbiol. Lett.* 66:203; use of *Streptococcus*: Barany *et al.* (1980) *J. Bacteriol.* 144:698;

Harlander (1987) "Transformation of *Streptococcus lactis* by electroporation, in: *Streptococcal Genetics* (ed. J. Ferretti and R. Curtiss III); Perry *et al.* (1981) *Infect. Immun.* 32:1295; Powell *et al.* (1988) *Appl. Environ. Microbiol.* 54:655; Somkuti *et al.* (1987) *Proc. 4th Evr. Cong. Biotechnology* 1:412.

v. Yeast Expression

Yeast expression systems are also known to one of ordinary skill in the art. A yeast promoter is any DNA sequence capable of binding yeast RNA polymerase and initiating the downstream (3') transcription of a coding sequence (e.g. structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site (the "TATA Box") and a transcription initiation site. A yeast promoter may also have a second domain called an upstream activator sequence (UAS), which, if present, is usually distal to the structural gene. The UAS permits regulated (inducible) expression. Constitutive expression occurs in the absence of a UAS. Regulated expression may be either positive or negative, thereby either enhancing or reducing transcription.

Yeast is a fermenting organism with an active metabolic pathway, therefore sequences encoding enzymes in the metabolic pathway provide particularly useful promoter sequences. Examples include alcohol dehydrogenase (ADH) (EPO Publ. No. 284 044), enolase, glucokinase, glucose-6-phosphate isomerase, glyceraldehyde-3-phosphate-dehydrogenase (GAP or GAPDH), hexokinase, phosphofructokinase, 3-phosphoglycerate mutase, and pyruvate kinase (PyK) (EPO Publ. No. 329 203). The yeast *PHO5* gene, encoding acid phosphatase, also provides useful promoter sequences (Myanohara *et al.* (1983) *Proc. Natl. Acad. Sci. USA* 80:1).

In addition, synthetic promoters which do not occur in nature also function as yeast promoters. For example, UAS sequences of one yeast promoter may be joined with the transcription activation region of another yeast promoter, creating a synthetic hybrid promoter. Examples of such hybrid promoters include the ADH regulatory sequence linked to the GAP transcription activation region (U.S. Patent Nos. 4,876,197 and 4,880,734). Other examples of hybrid promoters include promoters which consist of the regulatory sequences of

either the *ADH2*, *GAL4*, *GAL10*, OR *PHO5* genes, combined with the transcriptional activation region of a glycolytic enzyme gene such as GAP or PyK (EPO Publ. No. 164 556). Furthermore, a yeast promoter can include naturally occurring promoters of non-yeast origin that have the ability to bind yeast RNA polymerase and initiate transcription. Examples of such promoters include, *inter alia*, (Cohen *et al.* (1980) *Proc. Natl. Acad. Sci. USA* 77:1078; Henikoff *et al.* (1981) *Nature* 283:835; Hollenberg *et al.* (1981) *Curr. Topics Microbiol. Immunol.* 96:119; Hollenberg *et al.* (1979) "The Expression of Bacterial Antibiotic Resistance Genes in the Yeast *Saccharomyces cerevisiae*," in: *Plasmids of Medical, Environmental and Commercial Importance* (eds. K.N. Timmis and A. Puhler); Mercerau-Puigalon *et al.* (1980) *Gene* 11:163; Panthier *et al.* (1980) *Curr. Genet.* 2:109;).

A DNA molecule may be expressed intracellularly in yeast. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus of the recombinant protein will always be a methionine, which is encoded by the ATG start codon. If desired, methionine at the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide.

Fusion proteins provide an alternative for yeast expression systems, as well as in mammalian, plant, baculovirus, and bacterial expression systems. Usually, a DNA sequence encoding the N-terminal portion of an endogenous yeast protein, or other stable protein, is fused to the 5' end of heterologous coding sequences. Upon expression, this construct will provide a fusion of the two amino acid sequences. For example, the yeast or human superoxide dismutase (SOD) gene, can be linked at the 5' terminus of a foreign gene and expressed in yeast. The DNA sequence at the junction of the two amino acid sequences may or may not encode a cleavable site. See e.g., EPO Publ. No. 196056. Another example is a ubiquitin fusion protein. Such a fusion protein is made with the ubiquitin region that preferably retains a site for a processing enzyme (e.g. ubiquitin-specific processing protease) to cleave the ubiquitin from the foreign protein. Through this method, therefore, native foreign protein can be isolated (e.g., WO88/024066).

Alternatively, foreign proteins can also be secreted from the cell into the growth media by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provide for secretion in yeast of the foreign protein. Preferably, there are processing sites encoded between the leader fragment and the foreign gene that can

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be cleaved either *in vivo* or *in vitro*. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell.

DNA encoding suitable signal sequences can be derived from genes for secreted yeast proteins, such as the yeast invertase gene (EPO Publ. No. 012 873; JPO Publ. No. 62:096,086) and the A-factor gene (U.S. Patent 4,588,684). Alternatively, leaders of non-yeast origin, such as an interferon leader, exist that also provide for secretion in yeast (EPO Publ. No. 060 057).

A preferred class of secretion leaders are those that employ a fragment of the yeast alpha-factor gene, which contains both a "pre" signal sequence, and a "pro" region. The types of alpha-factor fragments that can be employed include the full-length pre-pro alpha factor leader (about 83 amino acid residues) as well as truncated alpha-factor leaders (usually about 25 to about 50 amino acid residues) (U.S. Patent Nos. 4,546,083 and 4,870,008; EPO Publ. No. 324 274). Additional leaders employing an alpha-factor leader fragment that provides for secretion include hybrid alpha-factor leaders made with a presequence of a first yeast, but a pro-region from a second yeast alpha factor. (See e.g., PCT Publ. No. WO 89/02463.)

Usually, transcription termination sequences recognized by yeast are regulatory regions located 3' to the translation stop codon, and thus together with the promoter flank the coding sequence. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Examples of transcription terminator sequence and other yeast-recognized termination sequences, such as those coding for glycolytic enzymes.

Usually, the above described components, comprising a promoter, leader (if desired), coding sequence of interest, and transcription termination sequence, are put together into expression constructs. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (e.g., plasmids) capable of stable maintenance in a host, such as yeast or bacteria. The replicon may have two replication systems, thus allowing it to be maintained, for example, in yeast for expression and in a prokaryotic host for cloning and amplification. Examples of such yeast-bacteria shuttle vectors include YEp24 (Botstein *et al.* (1979) *Gene* 8:17-24), pCI/1 (Brake *et al.* (1984) *Proc. Natl. Acad. Sci USA* 81:4642-4646), and YRp17 (Stinchcomb *et al.* (1982) *J. Mol. Biol.* 158:157). In addition, a replicon may be

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either a high or low copy number plasmid. A high copy number plasmid will generally have a copy number ranging from about 5 to about 200, and usually about 10 to about 150. A host containing a high copy number plasmid will preferably have at least about 10, and more preferably at least about 20. Enter a high or low copy number vector may be selected, depending upon the effect of the vector and the foreign protein on the host. See e.g., Brake *et al.*, *supra*.

Alternatively, the expression constructs can be integrated into the yeast genome with an integrating vector. Integrating vectors usually contain at least one sequence homologous to a yeast chromosome that allows the vector to integrate, and preferably contain two homologous sequences flanking the expression construct. Integrations appear to result from recombinations between homologous DNA in the vector and the yeast chromosome (Orr-Weaver *et al.* (1983) *Methods in Enzymol.* 101:228-245). An integrating vector may be directed to a specific locus in yeast by selecting the appropriate homologous sequence for inclusion in the vector. See Orr-Weaver *et al.*, *supra*. One or more expression construct may integrate, possibly affecting levels of recombinant protein produced (Rine *et al.* (1983) *Proc. Natl. Acad. Sci. USA* 80:6750). The chromosomal sequences included in the vector can occur either as a single segment in the vector, which results in the integration of the entire vector, or two segments homologous to adjacent segments in the chromosome and flanking the expression construct in the vector, which can result in the stable integration of only the expression construct.

Usually, extrachromosomal and integrating expression constructs may contain selectable markers to allow for the selection of yeast strains that have been transformed. Selectable markers may include biosynthetic genes that can be expressed in the yeast host, such as *ADE2*, *HIS4*, *LEU2*, *TRP1*, and *ALG7*, and the G418 resistance gene, which confer resistance in yeast cells to tunicamycin and G418, respectively. In addition, a suitable selectable marker may also provide yeast with the ability to grow in the presence of toxic compounds, such as metal. For example, the presence of *CUP1* allows yeast to grow in the presence of copper ions (Butt *et al.* (1987) *Microbiol. Rev.* 51:351).

Alternatively, some of the above described components can be put together into transformation vectors. Transformation vectors are usually comprised of a selectable marker

that is either maintained in a replicon or developed into an integrating vector, as described above.

Expression and transformation vectors, either extrachromosomal replicons or integrating vectors, have been developed for transformation into many yeasts. For example, expression vectors and methods of introducing exogenous DNA into yeast hosts have been developed for, *inter alia*, the following yeasts: *Candida albicans* (Kurtz, *et al.* (1986) *Mol. Cell. Biol.* 6:142); *Candida maltosa* (Kunze, *et al.* (1985) *J. Basic Microbiol.* 25:141); *Hansenula polymorpha* (Gleeson, *et al.* (1986) *J. Gen. Microbiol.* 132:3459; Roggenkamp *et al.* (1986) *Mol. Gen. Genet.* 202:302); *Kluyveromyces fragilis* (Das, *et al.* (1984) *J. Bacteriol.* 158:1165); *Kluyveromyces lactis* (De Louvencourt *et al.* (1983) *J. Bacteriol.* 154:737; Van den Berg *et al.* (1990) *Bio/Technology* 8:135); *Pichia guilliermondii* (Kunze *et al.* (1985) *J. Basic Microbiol.* 25:141); *Pichia pastoris* (Cregg, *et al.* (1985) *Mol. Cell. Biol.* 5:3376; U.S. Patent Nos. 4,837,148 and 4,929,555); *Saccharomyces cerevisiae* (Hinnen *et al.* (1978) *Proc. Natl. Acad. Sci. USA* 75:1929; Ito *et al.* (1983) *J. Bacteriol.* 153:163); *Schizosaccharomyces pombe* (Beach and Nurse (1981) *Nature* 300:706); and *Yarrowia lipolytica* (Davidow, *et al.* (1985) *Curr. Genet.* 10:380471 Gaillardin, *et al.* (1985) *Curr. Genet.* 10:49).

Methods of introducing exogenous DNA into yeast hosts are well-known in the art, and usually include either the transformation of spheroplasts or of intact yeast cells treated with alkali cations. Transformation procedures usually vary with the yeast species to be transformed. See e.g., [Kurtz *et al.* (1986) *Mol. Cell. Biol.* 6:142; Kunze *et al.* (1985) *J. Basic Microbiol.* 25:141; *Candida*]; [Gleeson *et al.* (1986) *J. Gen. Microbiol.* 132:3459; Roggenkamp *et al.* (1986) *Mol. Gen. Genet.* 202:302; *Hansenula*]; [Das *et al.* (1984) *J. Bacteriol.* 158:1165; De Louvencourt *et al.* (1983) *J. Bacteriol.* 154:1165; Van den Berg *et al.* (1990) *Bio/Technology* 8:135; *Kluyveromyces*]; [Cregg *et al.* (1985) *Mol. Cell. Biol.* 5:3376; Kunze *et al.* (1985) *J. Basic Microbiol.* 25:141; U.S. Patent Nos. 4,837,148 and 4,929,555; *Pichia*]; [Hinnen *et al.* (1978) *Proc. Natl. Acad. Sci. USA* 75:1929; Ito *et al.* (1983) *J. Bacteriol.* 153:163 *Saccharomyces*]; [Beach and Nurse (1981) *Nature* 300:706; *Schizosaccharomyces*]; [Davidow *et al.* (1985) *Curr. Genet.* 10:39; Gaillardin *et al.* (1985) *Curr. Genet.* 10:49; *Yarrowia*].

Definitions

A composition containing X is "substantially free of" Y when at least 85% by weight of the total X+Y in the composition is X. Preferably, X comprises at least about 90% by weight of the total of X+Y in the composition, more preferably at least about 95% or even 99% by weight.

The term "heterologous" refers to two biological components that are not found together in nature. The components may be host cells, genes, or regulatory regions, such as promoters. Although the heterologous components are not found together in nature, they can function together, as when a promoter heterologous to a gene is operably linked to the gene. Another example is where a Neisserial sequence is heterologous to a mouse host cell.

An "origin of replication" is a polynucleotide sequence that initiates and regulates replication of polynucleotides, such as an expression vector. The origin of replication behaves as an autonomous unit of polynucleotide replication within a cell, capable of replication under its own control. An origin of replication may be needed for a vector to replicate in a particular host cell. With certain origins of replication, an expression vector can be reproduced at a high copy number in the presence of the appropriate proteins within the cell. Examples of origins are the autonomously replicating sequences, which are effective in yeast; and the viral T-antigen, effective in COS-7 cells.

A "mutant" sequence is defined as a DNA, RNA or amino acid sequence differing from but having homology with the native or disclosed sequence. Depending on the particular sequence, the degree of homology between the native or disclosed sequence and the mutant sequence is preferably greater than 50% (e.g., 60%, 70%, 80%, 90%, 95%, 99% or more) which is calculated as described above. As used herein, an "allelic variant" of a nucleic acid molecule, or region, for which nucleic acid sequence is provided herein is a nucleic acid molecule, or region, that occurs at essentially the same locus in the genome of another or second isolate, and that, due to natural variation caused by, for example, mutation or recombination, has a similar but not identical nucleic acid sequence. A coding region allelic variant typically encodes a protein having similar activity to that of the protein encoded by the gene to which it is being compared. An allelic variant can also comprise an alteration in the 5' or 3' untranslated regions of the gene, such as in regulatory control regions. (see, for example, U.S. Patent 5,753,235).

Antibodies

As used herein, the term "antibody" refers to a polypeptide or group of polypeptides composed of at least one antibody combining site. An "antibody combining site" is the three-dimensional binding space with an internal surface shape and charge distribution complementary to the features of an epitope of an antigen, which allows a binding of the antibody with the antigen. "Antibody" includes, for example, vertebrate antibodies, hybrid antibodies, chimeric antibodies, humanized antibodies, altered antibodies, univalent antibodies, Fab proteins, and single domain antibodies.

Antibodies against the proteins of the invention are useful for affinity chromatography, immunoassays, and distinguishing/identifying *Neisseria* MenB proteins. Antibodies elicited against the proteins of the present invention bind to antigenic polypeptides or proteins or protein fragments that are present and specifically associated with strains of *Neisseria meningitidis* MenB. In some instances, these antigens may be associated with specific strains, such as those antigens specific for the MenB strains. The antibodies of the invention may be immobilized to a matrix and utilized in an immunoassay or on an affinity chromatography column, to enable the detection and/or separation of polypeptides, proteins or protein fragments or cells comprising such polypeptides, proteins or protein fragments. Alternatively, such polypeptides, proteins or protein fragments may be immobilized so as to detect antibodies bindably specific thereto.

Antibodies to the proteins of the invention, both polyclonal and monoclonal, may be prepared by conventional methods. In general, the protein is first used to immunize a suitable animal, preferably a mouse, rat, rabbit or goat. Rabbits and goats are preferred for the preparation of polyclonal sera due to the volume of serum obtainable, and the availability of labeled anti-rabbit and anti-goat antibodies. Immunization is generally performed by mixing or emulsifying the protein in saline, preferably in an adjuvant such as Freund's complete adjuvant, and injecting the mixture or emulsion parenterally (generally subcutaneously or intramuscularly). A dose of 50-200 µg/injection is typically sufficient. Immunization is generally boosted 2-6 weeks later with one or more injections of the protein in saline, preferably using Freund's incomplete adjuvant. One may alternatively generate antibodies by in vitro immunization using methods known in the art, which for the purposes of this

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invention is considered equivalent to *in vivo* immunization. Polyclonal antisera is obtained by bleeding the immunized animal into a glass or plastic container, incubating the blood at 25°C for one hour, followed by incubating at 4°C for 2-18 hours. The serum is recovered by centrifugation (e.g., 1,000g for 10 minutes). About 20-50 ml per bleed may be obtained from rabbits.

Monoclonal antibodies are prepared using the standard method of Kohler & Milstein (*Nature* (1975) 256:495-96), or a modification thereof. Typically, a mouse or rat is immunized as described above. However, rather than bleeding the animal to extract serum, the spleen (and optionally several large lymph nodes) is removed and dissociated into single cells. If desired, the spleen cells may be screened (after removal of nonspecifically adherent cells) by applying a cell suspension to a plate or well coated with the protein antigen. B-cells that express membrane-bound immunoglobulin specific for the antigen bind to the plate, and are not rinsed away with the rest of the suspension. Resulting B-cells, or all dissociated spleen cells, are then induced to fuse with myeloma cells to form hybridomas, and are cultured in a selective medium (e.g., hypoxanthine, aminopterin, thymidine medium, "HAT"). The resulting hybridomas are plated by limiting dilution, and are assayed for the production of antibodies which bind specifically to the immunizing antigen (and which do not bind to unrelated antigens). The selected MAb-secreting hybridomas are then cultured either *in vitro* (e.g., in tissue culture bottles or hollow fiber reactors), or *in vivo* (as ascites in mice).

If desired, the antibodies (whether polyclonal or monoclonal) may be labeled using conventional techniques. Suitable labels include fluorophores, chromophores, radioactive atoms (particularly ³²P and ¹²⁵I), electron-dense reagents, enzymes, and ligands having specific binding partners. Enzymes are typically detected by their activity. For example, horseradish peroxidase is usually detected by its ability to convert 3,3',5,5'-tetramethylbenzidine (TMB) to a blue pigment, quantifiable with a spectrophotometer. "Specific binding partner" refers to a protein capable of binding a ligand molecule with high specificity, as for example in the case of an antigen and a monoclonal antibody specific therefor. Other specific binding partners include biotin and avidin or streptavidin, IgG and protein A, and the numerous receptor-ligand couples known in the art. It should be understood that the above description is not meant to categorize the various

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labels into distinct classes, as the same label may serve in several different modes. For example, ^{125}I may serve as a radioactive label or as an electron-dense reagent. HRP may serve as enzyme or as antigen for a MAb. Further, one may combine various labels for desired effect. For example, MAbs and avidin also require labels in the practice of this invention: thus, one might label a MAb with biotin, and detect its presence with avidin labeled with ^{125}I , or with an anti-biotin MAb labeled with HRP. Other permutations and possibilities will be readily apparent to those of ordinary skill in the art, and are considered as equivalents within the scope of the instant invention.

Antigens, immunogens, polypeptides, proteins or protein fragments of the present invention elicit formation of specific binding partner antibodies. These antigens, immunogens, polypeptides, proteins or protein fragments of the present invention comprise immunogenic compositions of the present invention. Such immunogenic compositions may further comprise or include adjuvants, carriers, or other compositions that promote or enhance or stabilize the antigens, polypeptides, proteins or protein fragments of the present invention. Such adjuvants and carriers will be readily apparent to those of ordinary skill in the art.

Pharmaceutical Compositions

Pharmaceutical compositions can include either polypeptides, antibodies, or nucleic acid of the invention. The pharmaceutical compositions will comprise a therapeutically effective amount of either polypeptides, antibodies, or polynucleotides of the claimed invention.

The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent to treat, ameliorate, or prevent a desired disease or condition, or to exhibit a detectable therapeutic or preventative effect. The effect can be detected by, for example, chemical markers or antigen levels. Therapeutic effects also include reduction in physical symptoms, such as decreased body temperature, when given to a patient that is febrile. The precise effective amount for a subject will depend upon the subject's size and health, the nature and extent of the condition, and the therapeutics or combination of therapeutics selected for administration. Thus, it is not useful to specify an exact effective amount in

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advance. However, the effective amount for a given situation can be determined by routine experimentation and is within the judgment of the clinician.

For purposes of the present invention, an effective dose will be from about 0.01 mg/kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

A pharmaceutical composition can also contain a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent, such as antibodies or a polypeptide, genes, and other therapeutic agents. The term refers to any pharmaceutical carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition, and which may be administered without undue toxicity. Suitable carriers may be large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Such carriers are well known to those of ordinary skill in the art.

Pharmaceutically acceptable salts can be used therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of pharmaceutically acceptable excipients is available in Remington's Pharmaceutical Sciences (Mack Pub. Co., N.J. 1991).

Pharmaceutically acceptable carriers in therapeutic compositions may contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. Typically, the therapeutic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. Liposomes are included within the definition of a pharmaceutically acceptable carrier.

Delivery Methods

Once formulated, the compositions of the invention can be administered directly to the subject. The subjects to be treated can be animals; in particular, human subjects can be treated.

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Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, intravenously or intramuscularly or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal and transcutaneous applications, needles, and gene guns or hyposprays. Dosage treatment may be a single dose schedule or a multiple dose schedule.

Vaccines

Vaccines according to the invention may either be prophylactic (i.e., to prevent infection) or therapeutic (i.e., to treat disease after infection).

Such vaccines comprise immunizing antigen(s) or immunogen(s), immunogenic polypeptide, protein(s) or protein fragments, or nucleic acids (e.g., ribonucleic acid or deoxyribonucleic acid), usually in combination with "pharmaceutically acceptable carriers," which include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, lipid aggregates (such as oil droplets or liposomes), and inactive virus particles. Such carriers are well known to those of ordinary skill in the art. Additionally, these carriers may function as immunostimulating agents ("adjuvants"). Furthermore, the immunogen or antigen may be conjugated to a bacterial toxoid, such as a toxoid from diphtheria, tetanus, cholera, *H. pylori*, etc. pathogens.

Preferred adjuvants to enhance effectiveness of the composition include, but are not limited to: (1) aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate, aluminum sulfate, etc; (2) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) MF59 (PCT Publ. No. WO 90/14837), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing various amounts of MTP-PE (see below), although not required) formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, MA), (b) SAF, containing 10% Squalene, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP (see below) either microfluidized into a submicron emulsion or vortexed to generate a

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larger particle size emulsion, and (c) RibiTM adjuvant system (RAS), (Ribi Immunochem, Hamilton, MT) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (DetoxTM); (3) saponin adjuvants, such as StimulonTM (Cambridge Bioscience, Worcester, MA) may be used or particles generated therefrom such as ISCOMs (immunostimulating complexes); (4) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (5) cytokines, such as interleukins (e.g., IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, *etc.*), interferons (e.g., gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), *etc.*; (6) detoxified mutants of a bacterial ADP-ribosylating toxin such as a cholera toxin (CT), a pertussis toxin (PT), or an *E. coli* heat-labile toxin (LT), particularly LT-K63, LT-R72, CT-S109, PT-K9/G129; see, e.g., WO 93/13302 and WO 92/19265; and (7) other substances that act as immunostimulating agents to enhance the effectiveness of the composition. Alum and MF59 are preferred.

As mentioned above, muramyl peptides include, but are not limited to, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE), *etc.*

The vaccine compositions comprising immunogenic compositions (e.g., which may include the antigen, pharmaceutically acceptable carrier, and adjuvant) typically will contain diluents, such as water, saline, glycerol, ethanol, *etc.* Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. Alternatively, vaccine compositions comprising immunogenic compositions may comprise an antigen, polypeptide, protein, protein fragment or nucleic acid in a pharmaceutically acceptable carrier.

More specifically, vaccines comprising immunogenic compositions comprise an immunologically effective amount of the immunogenic polypeptides, as well as any other of the above-mentioned components, as needed. By "immunologically effective amount", it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and physical condition of the individual to be treated, the taxonomic group of

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individual to be treated (e.g., nonhuman primate, primate, *etc.*), the capacity of the individual's immune system to synthesize antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

Typically, the vaccine compositions or immunogenic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. The preparation also may be emulsified or encapsulated in liposomes for enhanced adjuvant effect, as discussed above under pharmaceutically acceptable carriers.

The immunogenic compositions are conventionally administered parenterally, e.g., by injection, either subcutaneously or intramuscularly. Additional formulations suitable for other modes of administration include oral and pulmonary formulations, suppositories, and transdermal and transcutaneous applications. Dosage treatment may be a single dose schedule or a multiple dose schedule. The vaccine may be administered in conjunction with other immunoregulatory agents.

As an alternative to protein-based vaccines, DNA vaccination may be employed (e.g., Robinson & Torres (1997) *Seminars in Immunology* 9:271-283; Donnelly *et al.* (1997) *Annu Rev Immunol* 15:617-648).

Gene Delivery Vehicles

Gene therapy vehicles for delivery of constructs, including a coding sequence of a therapeutic of the invention, to be delivered to the mammal for expression in the mammal, can be administered either locally or systemically. These constructs can utilize viral or non-viral vector approaches in *in vivo* or *ex vivo* modality. Expression of such coding sequence can be induced using endogenous mammalian or heterologous promoters. Expression of the coding sequence in vivo can be either constitutive or regulated.

The invention includes gene delivery vehicles capable of expressing the contemplated nucleic acid sequences. The gene delivery vehicle is preferably a viral vector and, more preferably, a retroviral, adenoviral, adeno-associated viral (AAV), herpes viral, or alphavirus vector. The viral vector can also be an astrovirus, coronavirus, orthomyxovirus, papovavirus,

paramyxovirus, parvovirus, picornavirus, poxvirus, or togavirus viral vector. See generally, Jolly (1994) *Cancer Gene Therapy* 1:51-64; Kimura (1994) *Human Gene Therapy* 5:845-852; Connelly (1995) *Human Gene Therapy* 6:185-193; and Kaplitt (1994) *Nature Genetics* 6:148-153.

Retroviral vectors are well known in the art, including B, C and D type retroviruses, xenotropic retroviruses (for example, NZB-X1, NZB-X2 and NZB9-1 (see O'Neill (1985) *J. Virol.* 53:160) polytropic retroviruses e.g., MCF and MCF-MLV (see Kelly (1983) *J. Virol.* 45:291), spumaviruses and lentiviruses. See RNA Tumor Viruses, Second Edition, Cold Spring Harbor Laboratory, 1985.

Portions of the retroviral gene therapy vector may be derived from different retroviruses. For example, retrovector LTRs may be derived from a Murine Sarcoma Virus, a tRNA binding site from a Rous Sarcoma Virus, a packaging signal from a Murine Leukemia Virus, and an origin of second strand synthesis from an Avian Leukosis Virus.

These recombinant retroviral vectors may be used to generate transduction competent retroviral vector particles by introducing them into appropriate packaging cell lines (see US patent 5,591,624). Retrovirus vectors can be constructed for site-specific integration into host cell DNA by incorporation of a chimeric integrase enzyme into the retroviral particle (see WO96/37626). It is preferable that the recombinant viral vector is a replication defective recombinant virus.

Packaging cell lines suitable for use with the above-described retrovirus vectors are well known in the art, are readily prepared (see WO95/30763 and WO92/05266), and can be used to create producer cell lines (also termed vector cell lines or "VCLs") for the production of recombinant vector particles. Preferably, the packaging cell lines are made from human parent cells (e.g., HT1080 cells) or mink parent cell lines, which eliminates inactivation in human serum.

Preferred retroviruses for the construction of retroviral gene therapy vectors include Avian Leukosis Virus, Bovine Leukemia Virus, Murine Leukemia Virus, Mink-Cell Focus-Inducing Virus, Murine Sarcoma Virus, Reticuloendotheliosis Virus and Rous Sarcoma Virus. Particularly preferred Murine Leukemia Viruses include 4070A and 1504A (Hartley and Rowe (1976) *J Virol* 19:19-25), Abelson (ATCC No. VR-999), Friend (ATCC No. VR-245), Graffi, Gross (ATCC No. VR-590), Kirsten, Harvey Sarcoma Virus and

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Rauscher (ATCC No. VR-998) and Moloney Murine Leukemia Virus (ATCC No. VR-190). Such retroviruses may be obtained from depositories or collections such as the American Type Culture Collection ("ATCC") in Rockville, Maryland or isolated from known sources using commonly available techniques.

Exemplary known retroviral gene therapy vectors employable in this invention include those described in patent applications GB2200651, EP0415731, EP0345242, EP0334301, WO89/02468; WO89/05349, WO89/09271, WO90/02806, WO90/07936, WO94/03622, WO93/25698, WO93/25234, WO93/11230, WO93/10218, WO91/02805, WO91/02825, WO95/07994, US 5,219,740, US 4,405,712, US 4,861,719, US 4,980,289, US 4,777,127, US 5,591,624. See also Vile (1993) *Cancer Res* 53:3860-3864; Vile (1993) *Cancer Res* 53:962-967; Ram (1993) *Cancer Res* 53 (1993) 83-88; Takamiya (1992) *J Neurosci Res* 33:493-503; Baba (1993) *J Neurosurg* 79:729-735; Mann (1983) *Cell* 33:153; Cane (1984) *Proc Natl Acad Sci* 81:6349; and Miller (1990) *Human Gene Therapy* 1.

Human adenoviral gene therapy vectors are also known in the art and employable in this invention. See, for example, Berkner (1988) *Biotechniques* 6:616 and Rosenfeld (1991) *Science* 252:431, and WO93/07283, WO93/06223, and WO93/07282. Exemplary known adenoviral gene therapy vectors employable in this invention include those described in the above referenced documents and in WO94/12649, WO93/03769, WO93/19191, WO94/28938, WO95/11984, WO95/00655, WO95/27071, WO95/29993, WO95/34671, WO96/05320, WO94/08026, WO94/11506, WO93/06223, WO94/24299, WO95/14102, WO95/24297, WO95/02697, WO94/28152, WO94/24299, WO95/09241, WO95/25807, WO95/05835, WO94/18922 and WO95/09654. Alternatively, administration of DNA linked to killed adenovirus as described in Curiel (1992) *Hum. Gene Ther.* 3:147-154 may be employed. The gene delivery vehicles of the invention also include adenovirus associated virus (AAV) vectors. Leading and preferred examples of such vectors for use in this invention are the AAV-2 based vectors disclosed in Srivastava, WO93/09239. Most preferred AAV vectors comprise the two AAV inverted terminal repeats in which the native D-sequences are modified by substitution of nucleotides, such that at least 5 native nucleotides and up to 18 native nucleotides, preferably at least 10 native nucleotides up to 18 native nucleotides, most preferably 10 native nucleotides are retained and the remaining nucleotides of the D-sequence are deleted or replaced with non-native nucleotides. The native

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D-sequences of the AAV inverted terminal repeats are sequences of 20 consecutive nucleotides in each AAV inverted terminal repeat (i.e., there is one sequence at each end) which are not involved in HP formation. The non-native replacement nucleotide may be any nucleotide other than the nucleotide found in the native D-sequence in the same position. Other employable exemplary AAV vectors are pWP-19, pWN-1, both of which are disclosed in Nahreini (1993) *Gene* 124:257-262. Another example of such an AAV vector is psub201 (see Samulski (1987) *J. Virol.* 61:3096). Another exemplary AAV vector is the Double-D ITR vector. Construction of the Double-D ITR vector is disclosed in US Patent 5,478,745. Still other vectors are those disclosed in Carter US Patent 4,797,368 and Muzyczka US Patent 5,139,941, Chartejee US Patent 5,474,935, and Kotin WO94/288157. Yet a further example of an AAV vector employable in this invention is SSV9AFABTKneo, which contains the AFP enhancer and albumin promoter and directs expression predominantly in the liver. Its structure and construction are disclosed in Su (1996) *Human Gene Therapy* 7:463-470. Additional AAV gene therapy vectors are described in US 5,354,678, US 5,173,414, US 5,139,941, and US 5,252,479.

The gene therapy vectors comprising sequences of the invention also include herpes vectors. Leading and preferred examples are herpes simplex virus vectors containing a sequence encoding a thymidine kinase polypeptide such as those disclosed in US 5,288,641 and EP0176170 (Roizman). Additional exemplary herpes simplex virus vectors include HFEM/ICP6-LacZ disclosed in WO95/04139 (Wistar Institute), pHSVlac described in Geller (1988) *Science* 241:1667-1669 and in WO90/09441 and WO92/07945, HSV Us3::pgC-lacZ described in Fink (1992) *Human Gene Therapy* 3:11-19 and HSV 7134, 2 RH 105 and GAL4 described in EP 0453242 (Breakefield), and those deposited with the ATCC as accession numbers ATCC VR-977 and ATCC VR-260.

Also contemplated are alpha virus gene therapy vectors that can be employed in this invention. Preferred alpha virus vectors are Sindbis viruses vectors. Togaviruses, Semliki Forest virus (ATCC VR-67; ATCC VR-1247), Middleberg virus (ATCC VR-370), Ross River virus (ATCC VR-373; ATCC VR-1246), Venezuelan equine encephalitis virus (ATCC VR923; ATCC VR-1250; ATCC VR-1249; ATCC VR-532), and those described in US patents 5,091,309, 5,217,879, and WO92/10578. More particularly, those alpha virus vectors described in U.S. Serial No. 08/405,627, filed March 15, 1995, WO94/21792, WO92/10578,

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WO95/07994, US 5,091,309 and US 5,217,879 are employable. Such alpha viruses may be obtained from depositories or collections such as the ATCC in Rockville, Maryland or isolated from known sources using commonly available techniques. Preferably, alphavirus vectors with reduced cytotoxicity are used (see USSN 08/679640).

DNA vector systems such as eukaryotic layered expression systems are also useful for expressing the nucleic acids of the invention. See WO95/07994 for a detailed description of eukaryotic layered expression systems. Preferably, the eukaryotic layered expression systems of the invention are derived from alphavirus vectors and most preferably from Sindbis viral vectors.

Other viral vectors suitable for use in the present invention include those derived from poliovirus, for example ATCC VR-58 and those described in Evans, *Nature* 339 (1989) 385 and Sabin (1973) *J. Biol. Standardization* 1:115; rhinovirus, for example ATCC VR-1110 and those described in Arnold (1990) *J Cell Biochem* L401; pox viruses such as canary pox virus or vaccinia virus, for example ATCC VR-111 and ATCC VR-2010 and those described in Fisher-Hoch (1989) *Proc Natl Acad Sci* 86:317; Flexner (1989) *Ann NY Acad Sci* 569:86, Flexner (1990) *Vaccine* 8:17; in US 4,603,112 and US 4,769,330 and WO89/01973; SV40 virus, for example ATCC VR-305 and those described in Mulligan (1979) *Nature* 277:108 and Madzak (1992) *J Gen Virol* 73:1533; influenza virus, for example ATCC VR-797 and recombinant influenza viruses made employing reverse genetics techniques as described in US 5,166,057 and in Enami (1990) *Proc Natl Acad Sci* 87:3802-3805; Enami & Palese (1991) *J Virol* 65:2711-2713 and Luytjes (1989) *Cell* 59:110, (see also McMichael (1983) *NEJ Med* 309:13, and Yap (1978) *Nature* 273:238 and *Nature* (1979) 277:108); human immunodeficiency virus as described in EP-0386882 and in Buchschacher (1992) *J. Virol.* 66:2731; measles virus, for example ATCC VR-67 and VR-1247 and those described in EP-0440219; Aura virus, for example ATCC VR-368; Bebaru virus, for example ATCC VR-600 and ATCC VR-1240; Cabassou virus, for example ATCC VR-922; Chikungunya virus, for example ATCC VR-64 and ATCC VR-1241; Fort Morgan Virus, for example ATCC VR-924; Getah virus, for example ATCC VR-369 and ATCC VR-1243; Kyzylagach virus, for example ATCC VR-927; Mayaro virus, for example ATCC VR-66; Mucambo virus, for example ATCC VR-580 and ATCC VR-1244; Ndumu virus, for example ATCC VR-371; Pixuna virus, for example ATCC VR-372 and ATCC VR-1245; Tonate virus, for example

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ATCC VR-925; Triniti virus, for example ATCC VR-469; Una virus, for example ATCC VR-374; Whataroa virus, for example ATCC VR-926; Y-62-33 virus, for example ATCC VR-375; O'Nyong virus, Eastern encephalitis virus, for example ATCC VR-65 and ATCC VR-1242; Western encephalitis virus, for example ATCC VR-70, ATCC VR-1251, ATCC VR-622 and ATCC VR-1252; and coronavirus, for example ATCC VR-740 and those described in Hamre (1966) *Proc Soc Exp Biol Med* 121:190.

Delivery of the compositions of this invention into cells is not limited to the above mentioned viral vectors. Other delivery methods and media may be employed such as, for example, nucleic acid expression vectors, polycationic condensed DNA linked or unlinked to killed adenovirus alone, for example see US Serial No. 08/366,787, filed December 30, 1994 and Curiel (1992) *Hum Gene Ther* 3:147-154 ligand linked DNA, for example see Wu (1989) *J Biol Chem* 264:16985-16987, eucaryotic cell delivery vehicles cells, for example see US Serial No. 08/240,030, filed May 9, 1994, and US Serial No. 08/404,796, deposition of photopolymerized hydrogel materials, hand-held gene transfer particle gun, as described in US Patent 5,149,655, ionizing radiation as described in US5,206,152 and in WO92/11033, nucleic charge neutralization or fusion with cell membranes. Additional approaches are described in Philip (1994) *Mol Cell Biol* 14:2411-2418 and in Woffendin (1994) *Proc Natl Acad Sci* 91:1581-1585.

Particle mediated gene transfer may be employed, for example see US Serial No. 60/023,867. Briefly, the sequence can be inserted into conventional vectors that contain conventional control sequences for high level expression, and then incubated with synthetic gene transfer molecules such as polymeric DNA-binding cations like polylysine, protamine, and albumin, linked to cell targeting ligands such as asialoorosomucoid, as described in Wu & Wu (1987) *J. Biol. Chem.* 262:4429-4432, insulin as described in Hucked (1990) *Biochem Pharmacol* 40:253-263, galactose as described in Plank (1992) *Bioconjugate Chem* 3:533-539, lactose or transferrin.

Naked DNA may also be employed to transform a host cell. Exemplary naked DNA introduction methods are described in WO 90/11092 and US 5,580,859. Uptake efficiency may be improved using biodegradable latex beads. DNA coated latex beads are efficiently transported into cells after endocytosis initiation by the beads. The method may be improved

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further by treatment of the beads to increase hydrophobicity and thereby facilitate disruption of the endosome and release of the DNA into the cytoplasm.

Liposomes that can act as gene delivery vehicles are described in U.S. 5,422,120, WO95/13796, WO94/23697, WO91/14445 and EP-524,968. As described in USSN. 60/023,867, on non-viral delivery, the nucleic acid sequences encoding a polypeptide can be inserted into conventional vectors that contain conventional control sequences for high level expression, and then be incubated with synthetic gene transfer molecules such as polymeric DNA-binding cations like polylysine, protamine, and albumin, linked to cell targeting ligands such as asialoorosomucoid, insulin, galactose, lactose, or transferrin. Other delivery systems include the use of liposomes to encapsulate DNA comprising the gene under the control of a variety of tissue-specific or ubiquitously-active promoters. Further non-viral delivery suitable for use includes mechanical delivery systems such as the approach described in Woffendin *et al* (1994) *Proc. Natl. Acad. Sci. USA* 91(24):11581-11585. Moreover, the coding sequence and the product of expression of such can be delivered through deposition of photopolymerized hydrogel materials. Other conventional methods for gene delivery that can be used for delivery of the coding sequence include, for example, use of hand-held gene transfer particle gun, as described in U.S. 5,149,655; use of ionizing radiation for activating transferred gene, as described in U.S. 5,206,152 and WO92/11033

Exemplary liposome and polycationic gene delivery vehicles are those described in US 5,422,120 and 4,762,915; in WO 95/13796; WO94/23697; and WO91/14445; in EP-0524968; and in Stryer, *Biochemistry*, pages 236-240 (1975) W.H. Freeman, San Francisco; Szoka (1980) *Biochem Biophys Acta* 600:1; Bayer (1979) *Biochem Biophys Acta* 550:464; Rivnay (1987) *Meth Enzymol* 149:119; Wang (1987) *Proc Natl Acad Sci* 84:7851; Plant (1989) *Anal Biochem* 176:420.

A polynucleotide composition can comprise a therapeutically effective amount of a gene therapy vehicle, as the term is defined above. For purposes of the present invention, an effective dose will be from about 0.01 mg/kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

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Delivery Methods

Once formulated, the polynucleotide compositions of the invention can be administered (1) directly to the subject; (2) delivered *ex vivo*, to cells derived from the subject; or (3) *in vitro* for expression of recombinant proteins. The subjects to be treated can be mammals or birds. Also, human subjects can be treated.

Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, transdermally or transcutaneously, intravenously or intramuscularly or delivered to the interstitial space of a tissue. The compositions can also be administered into a tumor or lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal applications, needles, and gene guns or hyposprays. Dosage treatment may be a single dose schedule or a multiple dose schedule. See WO98/20734.

Methods for the *ex vivo* delivery and reimplantation of transformed cells into a subject are known in the art and described in e.g., WO93/14778. Examples of cells useful in *ex vivo* applications include, for example, stem cells, particularly hematopoietic, lymph cells, macrophages, dendritic cells, or tumor cells.

Generally, delivery of nucleic acids for both *ex vivo* and *in vitro* applications can be accomplished by the following procedures, for example, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei, all well known in the art.

Polynucleotide and Polypeptide pharmaceutical compositions

In addition to the pharmaceutically acceptable carriers and salts described above, the following additional agents can be used with polynucleotide and/or polypeptide compositions.

A. Polypeptides

One example are polypeptides which include, without limitation: asialoorosomucoid (ASOR); transferrin; asialoglycoproteins; antibodies; antibody fragments; ferritin; interleukins; interferons, granulocyte, macrophage colony stimulating factor (GM-CSF),

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granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), stem cell factor and erythropoietin. Viral antigens, such as envelope proteins, can also be used. Also, proteins from other invasive organisms, such as the 17 amino acid peptide from the circumsporozoite protein of *Plasmodium falciparum* known as RII.

B. Hormones, Vitamins, Etc.

Other groups that can be included in a pharmaceutical composition include, for example: hormones, steroids, androgens, estrogens, thyroid hormone, or vitamins, folic acid.

C. Polyalkylenes, Polysaccharides, etc.

Also, polyalkylene glycol can be included in a pharmaceutical compositions with the desired polynucleotides and/or polypeptides. In a preferred embodiment, the polyalkylene glycol is polyethylene glycol. In addition, mono-, di-, or polysaccharides can be included. In a preferred embodiment of this aspect, the polysaccharide is dextran or DEAE-dextran. Also, chitosan and poly(lactide-co-glycolide) may be included in a pharmaceutical composition.

D. Lipids, and Liposomes

The desired polynucleotide or polypeptide can also be encapsulated in lipids or packaged in liposomes prior to delivery to the subject or to cells derived therefrom.

Lipid encapsulation is generally accomplished using liposomes which are able to stably bind or entrap and retain nucleic acid or polypeptide. The ratio of condensed polynucleotide to lipid preparation can vary but will generally be around 1:1 (mg DNA:micromoles lipid), or more of lipid. For a review of the use of liposomes as carriers for delivery of nucleic acids, see, Hug and Sleight (1991) *Biochim. Biophys. Acta.* 1097:1-17; Straubinger (1983) *Meth. Enzymol.* 101:512-527.

Liposomal preparations for use in the present invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner (1987) *Proc. Natl. Acad. Sci. USA* 84:7413-7416); mRNA (Malone (1989) *Proc. Natl. Acad. Sci. USA* 86:6077-6081); and purified transcription factors (Debs (1990) *J. Biol. Chem.* 265:10189-10192), in functional form.

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Cationic liposomes are readily available. For example, N(1-2,3-dioleoyloxy)propyl)-N,N,N-triethylammonium (DOTMA) liposomes are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, NY. (See, also, Felgner *supra*). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer). Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g., Szoka (1978) *Proc. Natl. Acad. Sci. USA* 75:4194-4198; WO90/11092 for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes.

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, AL), or can be easily prepared using readily available materials. Such materials include phosphatidyl choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

The liposomes can comprise multilammellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs). The various liposome-nucleic acid complexes are prepared using methods known in the art. See e.g., Straubinger (1983) *Meth. Immunol.* 101:512-527; Szoka (1978) *Proc. Natl. Acad. Sci. USA* 75:4194-4198; Papahadjopoulos (1975) *Biochim. Biophys. Acta* 394:483; Wilson (1979) *Cell* 17:77; Deamer & Bangham (1976) *Biochim. Biophys. Acta* 443:629; Ostro (1977) *Biochem. Biophys. Res. Commun.* 76:836; Fraley (1979) *Proc. Natl. Acad. Sci. USA* 76:3348; Enoch & Strittmatter (1979) *Proc. Natl. Acad. Sci. USA* 76:145; Fraley (1980) *J. Biol. Chem.* (1980) 255:10431; Szoka & Papahadjopoulos (1978) *Proc. Natl. Acad. Sci. USA* 75:145; and Schaefer-Ridder (1982) *Science* 215:166.

E. Lipoproteins

In addition, lipoproteins can be included with the polynucleotide or polypeptide to be delivered. Examples of lipoproteins to be utilized include: chylomicrons, HDL, IDL, LDL, and VLDL. Mutants, fragments, or fusions of these proteins can also be used. Also, modifications of naturally occurring lipoproteins can be used, such as acetylated LDL. These

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lipoproteins can target the delivery of polynucleotides to cells expressing lipoprotein receptors. Preferably, if lipoproteins are including with the polynucleotide to be delivered, no other targeting ligand is included in the composition.

Naturally occurring lipoproteins comprise a lipid and a protein portion. The protein portion are known as apoproteins. At the present, apoproteins A, B, C, D, and E have been isolated and identified. At least two of these contain several proteins, designated by Roman numerals, AI, AII, AIV; CI, CII, CIII.

A lipoprotein can comprise more than one apoprotein. For example, naturally occurring chylomicrons comprises of A, B, C, and E; over time these lipoproteins lose A and acquire C and E apoproteins. VLDL comprises A, B, C, and E apoproteins, LDL comprises apoprotein B; and HDL comprises apoproteins A, C, and E.

The amino acid sequences of these apoproteins are known and are described in, for example, Breslow (1985) *Annu Rev. Biochem* 54:699; Law (1986) *Adv. Exp Med. Biol.* 151:162; Chen (1986) *J Biol Chem* 261:12918; Kane (1980) *Proc Natl Acad Sci USA* 77:2465; and Utermann (1984) *Hum Genet* 65:232.

Lipoproteins contain a variety of lipids including, triglycerides, cholesterol (free and esters), and phospholipids. The composition of the lipids varies in naturally occurring lipoproteins. For example, chylomicrons comprise mainly triglycerides. A more detailed description of the lipid content of naturally occurring lipoproteins can be found, for example, in *Meth. Enzymol.* 128 (1986). The composition of the lipids are chosen to aid in conformation of the apoprotein for receptor binding activity. The composition of lipids can also be chosen to facilitate hydrophobic interaction and association with the polynucleotide binding molecule.

Naturally occurring lipoproteins can be isolated from serum by ultracentrifugation, for instance. Such methods are described in *Meth. Enzymol.* (*supra*); Pitas (1980) *J. Biochem.* 255:5454-5460 and Mahey (1979) *J Clin. Invest* 64:743-750.

Lipoproteins can also be produced by *in vitro* or recombinant methods by expression of the apoprotein genes in a desired host cell. See, for example, Atkinson (1986) *Annu Rev Biophys Chem* 15:403 and Radding (1958) *Biochim Biophys Acta* 30: 443.

Lipoproteins can also be purchased from commercial suppliers, such as Biomedical Technologies, Inc., Stoughton, Massachusetts, USA.

Further description of lipoproteins can be found in Zuckermann et al., PCT. Appln. No. US97/14465.

F. Polycationic Agents

Polycationic agents can be included, with or without lipoprotein, in a composition with the desired polynucleotide and/or polypeptide to be delivered.

Polycationic agents, typically, exhibit a net positive charge at physiological relevant pH and are capable of neutralizing the electrical charge of nucleic acids to facilitate delivery to a desired location. These agents have both in vitro, ex vivo, and in vivo applications. Polycationic agents can be used to deliver nucleic acids to a living subject either intramuscularly, subcutaneously, etc.

The following are examples of useful polypeptides as polycationic agents: polylysine, polyarginine, polyornithine, and protamine. Other examples of useful polypeptides include histones, protamines; human serum albumin, DNA binding proteins, non-histone chromosomal proteins, coat proteins from DNA viruses, such as Φ X174, transcriptional factors also contain domains that bind DNA and therefore may be useful as nucleic acid condensing agents. Briefly, transcriptional factors such as C/CEBP, c-jun, c-fos, AP-1, AP-2, AP-3, CPF, Prot-1, Sp-1, Oct-1, Oct-2, CREP, and TFIID contain basic domains that bind DNA sequences.

Organic polycationic agents include: spermine, spermidine, and putrescine.

The dimensions and of the physical properties of a polycationic agent can be extrapolated from the list above, to construct other polypeptide polycationic agents or to produce synthetic polycationic agents.

G. Synthetic Polycationic Agents

Synthetic polycationic agents which are useful in pharmaceutical compositions include, for example, DEAE-dextran, polybrene. Lipofectin™, and lipofectAMINE™ are monomers that form polycationic complexes when combined with polynucleotides or polypeptides.

Immunodiagnostic Assays

Neisseria MenB antigens, or antigenic fragments thereof, of the invention can be used in immunoassays to detect antibody levels (or, conversely, anti-*Neisseria* MenB antibodies can be used to detect antigen levels). Immunoassays based on well defined, recombinant antigens can be developed to replace invasive diagnostics methods. Antibodies to *Neisseria* MenB proteins or fragments thereof within biological samples, including for example, blood or serum samples, can be detected. Design of the immunoassays is subject to a great deal of variation, and a variety of these are known in the art. Protocols for the immunoassay may be based, for example, upon competition, or direct reaction, or sandwich type assays. Protocols may also, for example, use solid supports, or may be by immunoprecipitation. Most assays involve the use of labeled antibody or polypeptide; the labels may be, for example, fluorescent, chemiluminescent, radioactive, or dye molecules. Assays which amplify the signals from the probe are also known; examples of which are assays which utilize biotin and avidin, and enzyme-labeled and mediated immunoassays, such as ELISA assays.

Kits suitable for immunodiagnosis and containing the appropriate labeled reagents are constructed by packaging the appropriate materials, including the compositions of the invention, in suitable containers, along with the remaining reagents and materials (for example, suitable buffers, salt solutions, *etc.*) required for the conduct of the assay, as well as suitable set of assay instructions.

Nucleic Acid Hybridization

"Hybridization" refers to the association of two nucleic acid sequences to one another by hydrogen bonding. Typically, one sequence will be fixed to a solid support and the other will be free in solution. Then, the two sequences will be placed in contact with one another under conditions that favor hydrogen bonding. Factors that affect this bonding include: the type and volume of solvent; reaction temperature; time of hybridization; agitation; agents to block the non-specific attachment of the liquid phase sequence to the solid support (Denhardt's reagent or BLOTTO); concentration of the sequences; use of compounds to increase the rate of association of sequences (dextran sulfate or polyethylene glycol); and the

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stringency of the washing conditions following hybridization. See Sambrook *et al.* (*supra*) Volume 2, chapter 9, pages 9.47 to 9.57.

"Stringency" refers to conditions in a hybridization reaction that favor association of very similar sequences over sequences that differ. For example, the combination of temperature and salt concentration should be chosen that is approximately 120 to 200°C below the calculated T_m of the hybrid under study. The temperature and salt conditions can often be determined empirically in preliminary experiments in which samples of genomic DNA immobilized on filters are hybridized to the sequence of interest and then washed under conditions of different stringencies. See Sambrook *et al.* at page 9.50.

Variables to consider when performing, for example, a Southern blot are (1) the complexity of the DNA being blotted and (2) the homology between the probe and the sequences being detected. The total amount of the fragment(s) to be studied can vary a magnitude of 10, from 0.1 to 1 µg for a plasmid or phage digest to 10^{-9} to 10^{-8} g for a single copy gene in a highly complex eukaryotic genome. For lower complexity polynucleotides, substantially shorter blotting, hybridization, and exposure times, a smaller amount of starting polynucleotides, and lower specific activity of probes can be used. For example, a single-copy yeast gene can be detected with an exposure time of only 1 hour starting with 1 µg of yeast DNA, blotting for two hours, and hybridizing for 4-8 hours with a probe of 10^8 cpm/µg. For a single-copy mammalian gene a conservative approach would start with 10 µg of DNA, blot overnight, and hybridize overnight in the presence of 10% dextran sulfate using a probe of greater than 10^8 cpm/µg, resulting in an exposure time of ~24 hours.

Several factors can affect the melting temperature (T_m) of a DNA-DNA hybrid between the probe and the fragment of interest, and consequently, the appropriate conditions for hybridization and washing. In many cases the probe is not 100% homologous to the fragment. Other commonly encountered variables include the length and total G+C content of the hybridizing sequences and the ionic strength and formamide content of the hybridization buffer. The effects of all of these factors can be approximated by a single equation:

$$T_m = 81 + 16.6(\log_{10} C_i) + 0.4\%(G + C) - 0.6\%(\text{formamide}) - 600/n - 1.5\%(\text{mismatch})$$

where C_i is the salt concentration (monovalent ions) and n is the length of the hybrid in base pairs (slightly modified from Meinkoth & Wahl (1984) *Anal. Biochem.* 138:267-284).

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In designing a hybridization experiment, some factors affecting nucleic acid hybridization can be conveniently altered. The temperature of the hybridization and washes and the salt concentration during the washes are the simplest to adjust. As the temperature of the hybridization increases (i.e., stringency), it becomes less likely for hybridization to occur between strands that are nonhomologous, and as a result, background decreases. If the radiolabeled probe is not completely homologous with the immobilized fragment (as is frequently the case in gene family and interspecies hybridization experiments), the hybridization temperature must be reduced, and background will increase. The temperature of the washes affects the intensity of the hybridizing band and the degree of background in a similar manner. The stringency of the washes is also increased with decreasing salt concentrations.

In general, convenient hybridization temperatures in the presence of 50% formamide are 42°C for a probe with is 95% to 100% homologous to the target fragment, 37°C for 90% to 95% homology, and 32°C for 85% to 90% homology. For lower homologies, formamide content should be lowered and temperature adjusted accordingly, using the equation above. If the homology between the probe and the target fragment are not known, the simplest approach is to start with both hybridization and wash conditions which are nonstringent. If non-specific bands or high background are observed after autoradiography, the filter can be washed at high stringency and reexposed. If the time required for exposure makes this approach impractical, several hybridization and/or washing stringencies should be tested in parallel.

Nucleic Acid Probe Assays

Methods such as PCR, branched DNA probe assays, or blotting techniques utilizing nucleic acid probes according to the invention can determine the presence of cDNA or mRNA. A probe is said to "hybridize" with a sequence of the invention if it can form a duplex or double stranded complex, which is stable enough to be detected.

The nucleic acid probes will hybridize to the Neisserial nucleotide sequences of the invention (including both sense and antisense strands). Though many different nucleotide sequences will encode the amino acid sequence, the native Neisserial sequence is preferred because it is the actual sequence present in cells. mRNA represents a coding sequence and so

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a probe should be complementary to the coding sequence; single-stranded cDNA is complementary to mRNA, and so a cDNA probe should be complementary to the non-coding sequence.

The probe sequence need not be identical to the Neisserial sequence (or its complement) -- some variation in the sequence and length can lead to increased assay sensitivity if the nucleic acid probe can form a duplex with target nucleotides, which can be detected. Also, the nucleic acid probe can include additional nucleotides to stabilize the formed duplex. Additional Neisserial sequence may also be helpful as a label to detect the formed duplex. For example, a non-complementary nucleotide sequence may be attached to the 5' end of the probe, with the remainder of the probe sequence being complementary to a Neisserial sequence. Alternatively, non-complementary bases or longer sequences can be interspersed into the probe, provided that the probe sequence has sufficient complementarity with the a Neisserial sequence in order to hybridize therewith and thereby form a duplex which can be detected.

The exact length and sequence of the probe will depend on the hybridization conditions, such as temperature, salt condition and the like. For example, for diagnostic applications, depending on the complexity of the analyte sequence, the nucleic acid probe typically contains at least 10-20 nucleotides, preferably 15-25, and more preferably at least 30 nucleotides, although it may be shorter than this. Short primers generally require cooler temperatures to form sufficiently stable hybrid complexes with the template.

Probes may be produced by synthetic procedures, such as the triester method of Matteucci *et al.* (*J. Am. Chem. Soc.* (1981) 103:3185), or according to Urdea *et al.* (*Proc. Natl. Acad. Sci. USA* (1983) 80: 7461), or using commercially available automated oligonucleotide synthesizers.

The chemical nature of the probe can be selected according to preference. For certain applications, DNA or RNA are appropriate. For other applications, modifications may be incorporated e.g., backbone modifications, such as phosphorothioates or methylphosphonates, can be used to increase *in vivo* half-life, alter RNA affinity, increase nuclease resistance *etc.* (e.g., see Agrawal & Iyer (1995) *Curr Opin Biotechnol* 6:12-19; Agrawal (1996) *TIBTECH* 14:376-387); analogues such as peptide nucleic acids may also be

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used (e.g., see Corey (1997) *TIBTECH* 15:224-229; Buchardt *et al.* (1993) *TIBTECH* 11:384-386).

One example of a nucleotide hybridization assay is described by Urdea *et al.* in international patent application WO92/02526 (see also U.S. Patent 5,124,246).

Alternatively, the polymerase chain reaction (PCR) is another well-known means for detecting small amounts of target nucleic acids. The assay is described in: Mullis *et al.* (*Meth. Enzymol.* (1987) 155: 335-350); US patent 4,683,195; and US patent 4,683,202. Two "primer" nucleotides hybridize with the target nucleic acids and are used to prime the reaction. The primers can comprise sequence that does not hybridize to the sequence of the amplification target (or its complement) to aid with duplex stability or, for example, to incorporate a convenient restriction site. Typically, such sequence will flank the desired Neisserial sequence.

A thermostable polymerase creates copies of target nucleic acids from the primers using the original target nucleic acids as a template. After a threshold amount of target nucleic acids are generated by the polymerase, they can be detected by more traditional methods, such as Southern blots. When using the Southern blot method, the labeled probe will hybridize to the Neisserial sequence (or its complement).

Also, mRNA or cDNA can be detected by traditional blotting techniques described in Sambrook *et al.* (*supra*). mRNA, or cDNA generated from mRNA using a polymerase enzyme, can be purified and separated using gel electrophoresis. The nucleic acids on the gel are then blotted onto a solid support, such as nitrocellulose. The solid support is exposed to a labeled probe and then washed to remove any unhybridized probe. Next, the duplexes containing the labeled probe are detected. Typically, the probe is labeled with a radioactive moiety.

EXAMPLES

The invention is based on the 961 nucleotide sequences from the genome of *N. meningitidis* set out in Appendix C, SEQ ID NOs:1-961 of the '573 application, which together represent substantially the complete genome of serotype B of *N. meningitidis*, as well as the full length genome sequence shown in Appendix D, SEQ ID NO 1068 of the '573

application, and the full length genome sequence shown in Appendix A hereto, SEQ ID NO. 1.

It will be self-evident to the skilled person how this sequence information can be utilized according to the invention, as above described.

The standard techniques and procedures which may be employed in order to perform the invention (e.g. to utilize the disclosed sequences to predict polypeptides useful for vaccination or diagnostic purposes) were summarized above. This summary is not a limitation on the invention but, rather, gives examples that may be used, but are not required.

These sequences are derived from contigs shown in Appendix C (SEQ ID NOs 1-961) and from the full length genome sequence shown in Appendix D (SEQ ID NO 1068), which were prepared during the sequencing of the genome of *N. meningitidis* (strain B). The full length sequence was assembled using the TIGR Assembler as described by G.S. Sutton et al., *TIGR Assembler: A New Tool for Assembling Large Shotgun Sequencing Projects*, Genome Science and Technology, 1:9-19 (1995) [see also R. D. Fleischmann, et al., Science 269, 496-512 (1995); C. M. Fraser, et al., Science 270, 397-403 (1995); C. J. Bult, et al., Science 273, 1058-73 (1996); C. M. Fraser, et. al, Nature 390, 580-586 (1997); J.-F. Tomb, et. al., Nature 388, 539-547 (1997); H. P. Klenk, et al., Nature 390, 364-70 (1997); C. M. Fraser, et al., Science 281, 375-88 (1998); M. J. Gardner, et al., Science 282, 1126-1132 (1998); K. E. Nelson, et al., Nature 399, 323-9 (1999)]. Then, using the above-described methods, putative translation products of the sequences were determined. Computer analysis of the translation products were determined based on database comparisons. Corresponding gene and protein sequences, if any, were identified in *Neisseria meningitidis* (Strain A) and *Neisseria gonorrhoeae*. Then the proteins were expressed, purified, and characterized to assess their antigenicity and immunogenicity.

In particular, the following methods were used to express, purify, and biochemically characterize the proteins of the invention.

Chromosomal DNA Preparation

N. meningitidis strain 2996 was grown to exponential phase in 100 ml of GC medium, harvested by centrifugation, and resuspended in 5 ml buffer (20% Sucrose, 50 mM Tris-HCl, 50 mM EDTA, adjusted to pH 8.0). After 10 minutes incubation on ice, the bacteria were

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lysed by adding 10 ml lysis solution (50 mM NaCl, 1% Na-Sarkosyl, 50 µg/ml Proteinase K), and the suspension was incubated at 37°C for 2 hours. Two phenol extractions (equilibrated to pH 8) and one CHCl_3 /isoamylalcohol (24:1) extraction were performed. DNA was precipitated by addition of 0.3M sodium acetate and 2 volumes ethanol, and was collected by centrifugation. The pellet was washed once with 70% ethanol and redissolved in 4 ml buffer (10 mM Tris-HCl, 1mM EDTA, pH 8). The DNA concentration was measured by reading the OD at 260 nm.

Oligonucleotide design

Synthetic oligonucleotide primers were designed on the basis of the coding sequence of each ORF, using (a) the meningococcus B sequence when available, or (b) the gonococcus/meningococcus A sequence, adapted to the codon preference usage of meningococcus. Any predicted signal peptides were omitted, by deducing the 5'-end amplification primer sequence immediately downstream from the predicted leader sequence.

For most ORFs, the 5' primers included two restriction enzyme recognition sites (*Bam*HI-*Nde*I, *Bam*HI-*Nhe*I, or *Eco*RI-*Nhe*I, depending on the gene's restriction pattern); the 3' primers included a *Xho*I restriction site. This procedure was established in order to direct the cloning of each amplification product (corresponding to each ORF) into two different expression systems: pGEX-KG (using either *Bam*HI-*Xho*I or *Eco*RI-*Xho*I), and pET21b+ (using either *Nde*I-*Xho*I or *Nhe*I-*Xho*I).

5'-end primer tail:	<u>CGCGGATCCC</u> <u>CATATG</u>	(<i>Bam</i> HI- <i>Nde</i> I)
	<u>CGCGGATCCGCTAGC</u>	(<i>Bam</i> HI- <i>Nhe</i> I)
	<u>CCGGAATTCTAGCTAGC</u>	(<i>Eco</i> RI- <i>Nhe</i> I)
3'-end primer tail:	<u>CCCGCTCGAG</u>	(<i>Xho</i> I)

For some ORFs, two different amplifications were performed to clone each ORF in the two expression systems. Two different 5' primers were used for each ORF; the same 3' *Xho*I primer was used as before:

5'-end primer tail:	<u>GGAATTCC</u> <u>CATATG</u> <u>GCCATGG</u>	(<i>Nde</i> I)
5'-end primer tail:	<u>CGGGATCC</u>	(<i>Bam</i> HI)

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Other ORFs were cloned in the pTRC expression vector and expressed as an amino-terminus His-tag fusion. The predicted signal peptide may be included in the final product. *NheI*-*BamHI* restriction sites were incorporated using primers:

5'-end primer tail: GATCAGCTAGCCATATG (*NheI*)

3'-end primer tail: CGGGATCC (*BamHI*)

As well as containing the restriction enzyme recognition sequences, the primers included nucleotides which hybridized to the sequence to be amplified. The number of hybridizing nucleotides depended on the melting temperature of the whole primer, and was determined for each primer using the formulae:

$$T_m = 4 (G+C) + 2 (A+T) \quad (\text{tail excluded})$$

$$T_m = 64.9 + 0.41 (\% \text{ GC}) - 600/N \quad (\text{whole primer})$$

The average melting temperature of the selected oligos were 65-70°C for the whole oligo and 50-55°C for the hybridising region alone.

Oligos were synthesized by a Perkin Elmer 394 DNA/RNA Synthesizer, eluted from the columns in 2 ml $\text{NH}_4\text{-OH}$, and deprotected by 5 hours incubation at 56 °C. The oligos were precipitated by addition of 0.3M Na-Acetate and 2 volumes ethanol. The samples were then centrifuged and the pellets resuspended in either 100µl or 1ml of water. OD_{260} was determined using a Perkin Elmer Lambda Bio spectrophotometer and the concentration was determined and adjusted to 2-10 pmol/µl.

Table 1 shows the forward and reverse primers used for each amplification. In certain cases, it might be noted that the sequence of the primer does not exactly match the sequence in the ORF. When initial amplifications are performed, the complete 5' and/or 3' sequence may not be known for some meningococcal ORFs, although the corresponding sequences may have been identified in gonococcus. For amplification, the gonococcal sequences could thus be used as the basis for primer design, altered to take account of codon preference. In particular, the following codons may be changed: ATA→ATT; TCG→TCT; CAG→CAA; AAG→AAA; GAG→GAA; CGA and CGG→CGC; GGG→GGC.

Amplification

The standard PCR protocol was as follows: 50-200 ng of genomic DNA were used as a template in the presence of 20-40 µM of each oligo, 400-800 µM dNTPs solution, 1x PCR

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buffer (including 1.5 mM MgCl₂), 2.5 units *TaqI* DNA polymerase (using Perkin-Elmer AmpliTaq, GIBCO Platinum, Pwo DNA polymerase, or Tahara Shuzo Taq polymerase).

In some cases, PCR was optimised by the addition of 10µl DMSO or 50 µl 2M betaine.

After a hot start (adding the polymerase during a preliminary 3 minute incubation of the whole mix at 95°C), each sample underwent a double-step amplification: the first 5 cycles were performed using as the hybridization temperature the one of the oligos excluding the restriction enzymes tail, followed by 30 cycles performed according to the hybridization temperature of the whole length oligos. The cycles were followed by a final 10 minute extension step at 72°C.

The standard cycles were as follows:

	Denaturation	Hybridisation	Elongation
First 5 cycles	30 seconds 95°C	30 seconds 50-55°C	30-60 seconds 72°C
Last 30 cycles	30 seconds 95°C	30 seconds 65-70°C	30-60 seconds 72°C

The elongation time varied according to the length of the ORF to be amplified.

The amplifications were performed using either a 9600 or a 2400 Perkin Elmer GeneAmp PCR System. To check the results, 1/10 of the amplification volume was loaded onto a 1-1.5% agarose gel and the size of each amplified fragment compared with a DNA molecular weight marker.

The amplified DNA was either loaded directly on a 1% agarose gel or first precipitated with ethanol and resuspended in a suitable volume to be loaded on a 1% agarose gel. The DNA fragment corresponding to the right size band was then eluted and purified from gel, using the Qiagen Gel Extraction Kit, following the instructions of the manufacturer. The final volume of the DNA fragment was 30µl or 50µl of either water or 10mM Tris, pH 8.5.

Digestion of PCR fragments

The purified DNA corresponding to the amplified fragment was split into 2 aliquots and double-digested with:

NdeI/XhoI or *NheI/XhoI* for cloning into pET-21b+ and further expression of the protein as a C-terminus His-tag fusion

BamHI/XhoI or *EcoRI/XhoI* for cloning into pGEX-KG and further expression of the protein as a GST N-terminus fusion.

For ORF 76, *NheI/BamHI* for cloning into pTRC-HisA vector and further expression of the protein as N-terminus His-tag fusion.

Each purified DNA fragment was incubated (37°C for 3 hours to overnight) with 20 units of each restriction enzyme (New England Biolabs) in a either 30 or 40 µl final volume in the presence of the appropriate buffer. The digestion product was then purified using the QIAquick PCR purification kit, following the manufacturer's instructions, and eluted in a final volume of 30 (or 50) µl of either water or 10mM Tris-HCl, pH 8.5. The final DNA concentration was determined by 1% agarose gel electrophoresis in the presence of titrated molecular weight marker.

Digestion of the cloning vectors (pET22B, pGEX-KG and pTRC-His A)

10 µg plasmid was double-digested with 50 units of each restriction enzyme in 200 µl reaction volume in the presence of appropriate buffer by overnight incubation at 37°C. After loading the whole digestion on a 1% agarose gel, the band corresponding to the digested vector was purified from the gel using the Qiagen QIAquick Gel Extraction Kit and the DNA was eluted in 50 µl of 10 mM Tris-HCl, pH 8.5. The DNA concentration was evaluated by measuring OD₂₆₀ of the sample, and adjusted to 50 µg/µl. 1 µl of plasmid was used for each cloning procedure.

Cloning

The fragments corresponding to each ORF, previously digested and purified, were ligated in both pET22b and pGEX-KG. In a final volume of 20 µl, a molar ratio of 3:1 fragment/vector was ligated using 0.5 µl of NEB T4 DNA ligase (400 units/µl), in the presence of the buffer supplied by the manufacturer. The reaction was incubated at room temperature for 3 hours. In some experiments, ligation was performed using the Boehringer "Rapid Ligation Kit", following the manufacturer's instructions.

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In order to introduce the recombinant plasmid in a suitable strain, 100 µl *E. coli* DH5 competent cells were incubated with the ligase reaction solution for 40 minutes on ice, then at 37°C for 3 minutes, then, after adding 800 µl LB broth, again at 37°C for 20 minutes. The cells were then centrifuged at maximum speed in an Eppendorf microfuge and resuspended in approximately 200 µl of the supernatant. The suspension was then plated on LB ampicillin (100 mg/ml).

The screening of the recombinant clones was performed by growing 5 randomly-chosen colonies overnight at 37 °C in either 2 ml (pGEX or pTC clones) or 5ml (pET clones) LB broth + 100 µg/ml ampicillin. The cells were then pelleted and the DNA extracted using the Qiagen QIAprep Spin Miniprep Kit, following the manufacturer's instructions, to a final volume of 30 µl. 5 µl of each individual miniprep (approximately 1g) were digested with either *NdeI/XhoI* or *BamHI/XhoI* and the whole digestion loaded onto a 1-1.5% agarose gel (depending on the expected insert size), in parallel with the molecular weight marker (1Kb DNA Ladder, GIBCO). The screening of the positive clones was made on the base of the correct insert size.

Cloning

Certain ORFs may be cloned into the pGEX-HIS vector using *EcoRI-PstI*, *EcoRI-SalI*, or *SalI-PstI* cloning sites. After cloning, the recombinant plasmids may be introduced in the *E.coli* host W3110.

Expression

Each ORF cloned into the expression vector may then be transformed into the strain suitable for expression of the recombinant protein product. 1 µl of each construct was used to transform 30 µl of *E.coli* BL21 (pGEX vector), *E.coli* TOP 10 (pTRC vector) or *E.coli* BL21-DE3 (pET vector), as described above. In the case of the pGEX-His vector, the same *E.coli* strain (W3110) was used for initial cloning and expression. Single recombinant colonies were inoculated into 2ml LB+Amp (100 µg/ml), incubated at 37°C overnight, then diluted 1:30 in 20 ml of LB+Amp (100 µg/ml) in 100 ml flasks, making sure that the OD₆₀₀ ranged between 0.1 and 0.15. The flasks were incubated at 30°C into gyratory water bath shakers until OD indicated exponential growth suitable for induction of expression (0.4-0.8 OD for

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pET and pTRC vectors; 0.8-1 OD for pGEX and pGEX-His vectors). For the pET, pTRC and pGEX-His vectors, the protein expression was induced by addition of 1mM IPTG, whereas in the case of pGEX system the final concentration of IPTG was 0.2 mM. After 3 hours incubation at 30°C, the final concentration of the sample was checked by OD. In order to check expression, 1ml of each sample was removed, centrifuged in a microfuge, the pellet resuspended in PBS, and analysed by 12% SDS-PAGE with Coomassie Blue staining. The whole sample was centrifuged at 6000g and the pellet resuspended in PBS for further use.

GST-fusion proteins large-scale purification.

A single colony was grown overnight at 37°C on LB+Amp agar plate. The bacteria were inoculated into 20 ml of LB+Amp liquid culture in a water bath shaker and grown overnight. Bacteria were diluted 1:30 into 600 ml of fresh medium and allowed to grow at the optimal temperature (20-37°C) to OD₅₅₀ 0.8-1. Protein expression was induced with 0.2mM IPTG followed by three hours incubation. The culture was centrifuged at 8000 rpm at 4°C. The supernatant was discarded and the bacterial pellet was resuspended in 7.5 ml cold PBS. The cells were disrupted by sonication on ice for 30 sec at 40W using a Branson sonifier B-15, frozen and thawed two times and centrifuged again. The supernatant was collected and mixed with 150µl Glutathione-Sepharose 4B resin (Pharmacia) (previously washed with PBS) and incubated at room temperature for 30 minutes. The sample was centrifuged at 700g for 5 minutes at 4°C. The resin was washed twice with 10 ml cold PBS for 10 minutes, resuspended in 1ml cold PBS, and loaded on a disposable column. The resin was washed twice with 2ml cold PBS until the flow-through reached OD₂₈₀ of 0.02-0.06. The GST-fusion protein was eluted by addition of 700µl cold Glutathione elution buffer 10mM reduced glutathione, 50mM Tris-HCl) and fractions collected until the OD₂₈₀ was 0.1. 21µl of each fraction were loaded on a 12% SDS gel using either Biorad SDS-PAGE Molecular weight standard broad range (M1) (200, 116.25, 97.4, 66.2, 45, 31, 21.5, 14.4, 6.5 kDa) or Amersham Rainbow Marker (M") (220, 66, 46, 30, 21.5, 14.3 kDa) as standards. As the MW of GST is 26kDa, this value must be added to the MW of each GST-fusion protein.

His-fusion soluble proteins large-scale purification.

A single colony was grown overnight at 37°C on a LB + Amp agar plate. The bacteria were inoculated into 20ml of LB+Amp liquid culture and incubated overnight in a water bath shaker. Bacteria were diluted 1:30 into 600ml fresh medium and allowed to grow at the optimal temperature (20-37°C) to OD₅₅₀ 0.6-0.8. Protein expression was induced by addition of 1 mM IPTG and the culture further incubated for three hours. The culture was centrifuged at 8000 rpm at 4°C, the supernatant was discarded and the bacterial pellet was resuspended in 7.5ml cold 10mM imidazole buffer (300 mM NaCl, 50 mM phosphate buffer, 10 mM imidazole, pH 8). The cells were disrupted by sonication on ice for 30 sec at 40W using a Branson sonifier B-15, frozen and thawed two times and centrifuged again. The supernatant was collected and mixed with 150µl Ni²⁺-resin (Pharmacia) (previously washed with 10mM imidazole buffer) and incubated at room temperature with gentle agitation for 30 minutes. The sample was centrifuged at 700g for 5 minutes at 4°C. The resin was washed twice with 10 ml cold 10mM imidazole buffer for 10 minutes, resuspended in 1ml cold 10mM imidazole buffer and loaded on a disposable column. The resin was washed at 4°C with 2ml cold 10mM imidazole buffer until the flow-through reached the O.D₂₈₀ of 0.02-0.06. The resin was washed with 2ml cold 20mM imidazole buffer (300 mM NaCl, 50 mM phosphate buffer, 20 mM imidazole, pH 8) until the flow-through reached the O.D₂₈₀ of 0.02-0.06. The His-fusion protein was eluted by addition of 700µl cold 250mM imidazole buffer (300 mM NaCl, 50 mM phosphate buffer, 250 mM imidazole, pH 8) and fractions collected until the O.D₂₈₀ was 0.1. 21µl of each fraction were loaded on a 12% SDS gel.

His-fusion insoluble proteins large-scale purification.

A single colony was grown overnight at 37 °C on a LB + Amp agar plate. The bacteria were inoculated into 20 ml of LB+Amp liquid culture in a water bath shaker and grown overnight. Bacteria were diluted 1:30 into 600ml fresh medium and let to grow at the optimal temperature (37°C) to O.D₅₅₀ 0.6-0.8. Protein expression was induced by addition of 1 mM IPTG and the culture further incubated for three hours. The culture was centrifuged at 8000rpm at 4°C. The supernatant was discarded and the bacterial pellet was resuspended in 7.5 ml buffer B (urea 8M, 10mM Tris-HCl, 100mM phosphate buffer, pH 8.8). The cells were disrupted by sonication on ice for 30 sec at 40W using a Branson sonifier B-15, frozen

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and thawed twice and centrifuged again. The supernatant was stored at -20°C, while the pellets were resuspended in 2 ml guanidine buffer (6M guanidine hydrochloride, 100mM phosphate buffer, 10 mM Tris-HCl, pH 7.5) and treated in a homogenizer for 10 cycles. The product was centrifuged at 13000 rpm for 40 minutes. The supernatant was mixed with 150µl Ni²⁺-resin (Pharmacia) (previously washed with buffer B) and incubated at room temperature with gentle agitation for 30 minutes. The sample was centrifuged at 700 g for 5 minutes at 4°C. The resin was washed twice with 10 ml buffer B for 10 minutes, resuspended in 1ml buffer B, and loaded on a disposable column. The resin was washed at room temperature with 2ml buffer B until the flow-through reached the OD₂₈₀ of 0.02-0.06. The resin was washed with 2ml buffer C (urea 8M, 10mM Tris-HCl, 100mM phosphate buffer, pH 6.3) until the flow-through reached the O.D₂₈₀ of 0.02-0.06. The His-fusion protein was eluted by addition of 700µl elution buffer (urea 8M, 10mM Tris-HCl, 100mM phosphate buffer, pH 4.5) and fractions collected until the OD₂₈₀ was 0.1. 21µl of each fraction were loaded on a 12% SDS gel.

His-fusion proteins renaturation

10% glycerol was added to the denatured proteins. The proteins were then diluted to 20µg/ml using dialysis buffer I (10% glycerol, 0.5M arginine, 50mM phosphate buffer, 5mM reduced glutathione, 0.5mM oxidised glutathione, 2M urea, pH 8.8) and dialysed against the same buffer at 4°C for 12-14 hours. The protein was further dialysed against dialysis buffer II (10% glycerol, 0.5M arginine, 50mM phosphate buffer, 5mM reduced glutathione, 0.5mM oxidised glutathione, pH 8.8) for 12-14 hours at 4°C. Protein concentration was evaluated using the formula:

$$\text{Protein (mg/ml)} = (1.55 \times \text{OD}_{280}) - (0.76 \times \text{OD}_{260})$$

Mice immunisations

20µg of each purified protein were used to immunise mice intraperitoneally. In the case of some ORFs, Balb-C mice were immunised with Al(OH)₃ as adjuvant on days 1, 21 and 42, and immune response was monitored in samples taken on day 56. For other ORFs, CD1 mice could be immunised using the same protocol. For other ORFs, CD1 mice could be immunised using Freund's adjuvant, and the same immunisation protocol was used, except that the immune response was measured on day 42, rather than 56. Similarly, for still other

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ORFs, CD1 mice could be immunised with Freund's adjuvant, but the immune response was measured on day 49.

ELISA assay (sera analysis)

The acapsulated MenB M7 strain was plated on chocolate agar plates and incubated overnight at 37°C. Bacterial colonies were collected from the agar plates using a sterile dracon swab and inoculated into 7ml of Mueller-Hinton Broth (Difco) containing 0.25% Glucose. Bacterial growth was monitored every 30 minutes by following OD₆₂₀. The bacteria were let to grow until the OD reached the value of 0.3-0.4. The culture was centrifuged for 10 minutes at 10000 rpm. The supernatant was discarded and bacteria were washed once with PBS, resuspended in PBS containing 0.025% formaldehyde, and incubated for 2 hours at room temperature and then overnight at 4°C with stirring. 100µl bacterial cells were added to each well of a 96 well Greiner plate and incubated overnight at 4°C. The wells were then washed three times with PBT washing buffer (0.1% Tween-20 in PBS). 200 µl of saturation buffer (2.7% Polyvinylpyrrolidone 10 in water) was added to each well and the plates incubated for 2 hours at 37°C. Wells were washed three times with PBT. 200 µl of diluted sera (Dilution buffer: 1% BSA, 0.1% Tween-20, 0.1% NaN₃ in PBS) were added to each well and the plates incubated for 90 minutes at 37°C. Wells were washed three times with PBT. 100 µl of HRP-conjugated rabbit anti-mouse (Dako) serum diluted 1:2000 in dilution buffer were added to each well and the plates were incubated for 90 minutes at 37°C. Wells were washed three times with PBT buffer. 100 µl of substrate buffer for HRP (25 ml of citrate buffer pH5, 10 mg of O-phenildiamine and 10 µl of H₂O) were added to each well and the plates were left at room temperature for 20 minutes. 100 µl H₂SO₄ was added to each well and OD₄₉₀ was followed. The ELISA was considered positive when OD₄₉₀ was 2.5 times the respective pre-immune sera.

FACScan bacteria Binding Assay procedure.

The acapsulated MenB M7 strain was plated on chocolate agar plates and incubated overnight at 37°C. Bacterial colonies were collected from the agar plates using a sterile dracon swab and inoculated into 4 tubes containing 8ml each Mueller-Hinton Broth (Difco) containing 0.25% glucose. Bacterial growth was monitored every 30 minutes by following

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OD₆₂₀. The bacteria were let to grow until the OD reached the value of 0.35-0.5. The culture was centrifuged for 10 minutes at 4000 rpm. The supernatant was discarded and the pellet was resuspended in blocking buffer (1% BSA, 0.4% NaN₃) and centrifuged for 5 minutes at 4000 rpm. Cells were resuspended in blocking buffer to reach OD₆₂₀ of 0.07. 100µl bacterial cells were added to each well of a Costar 96 well plate. 100µl of diluted (1:200) sera (in blocking buffer) were added to each well and plates incubated for 2 hours at 4°C. Cells were centrifuged for 5 minutes at 4000 rpm, the supernatant aspirated and cells washed by addition of 200µl/well of blocking buffer in each well. 100µl of R-Phicoerytrin conjugated F(ab)₂ goat anti-mouse, diluted 1:100, was added to each well and plates incubated for 1 hour at 4°C. Cells were spun down by centrifugation at 4000rpm for 5 minutes and washed by addition of 200µl/well of blocking buffer. The supernatant was aspirated and cells resuspended in 200µl/well of PBS, 0.25% formaldehyde. Samples were transferred to FACScan tubes and read. The condition for FACScan setting were: FL1 on, FL2 and FL3 off; FSC-H Treshold:92; FSC PMT Voltage: E 02; SSC PMT: 474; Amp. Gains 7.1; FL-2 PMT: 539. Compensation values: 0.

OMV preparations

Bacteria were grown overnight on 5 GC plates, harvested with a loop and resuspended in 10 ml 20mM Tris-HCl. Heat inactivation was performed at 56°C for 30 minutes and the bacteria disrupted by sonication for 10' on ice (50% duty cycle, 50% output). Unbroken cells were removed by centrifugation at 5000g for 10 minutes and the total cell envelope fraction recovered by centrifugation at 50000g at 4°C for 75 minutes. To extract cytoplasmic membrane proteins from the crude outer membranes, the whole fraction was resuspended in 2% sarkosyl (Sigma) and incubated at room temperature for 20 minutes. The suspension was centrifuged at 10000g for 10 minutes to remove aggregates, and the supernatant further ultracentrifuged at 50000g for 75 minutes to pellet the outer membranes. The outer membranes were resuspended in 10mM Tris-HCl, pH8 and the protein concentration measured by the Bio-Rad Protein assay, using BSA as a standard.

Whole Extracts preparation

Bacteria were grown overnight on a GC plate, harvested with a loop and resuspended in 1ml of 20mM Tris-HCl. Heat inactivation was performed at 56°C for 30' minutes.

Western blotting

Purified proteins (500ng/lane), outer membrane vesicles (5 µg) and total cell extracts (25µg) derived from MenB strain 2996 were loaded on 15% SDS-PAGE and transferred to a nitrocellulose membrane. The transfer was performed for 2 hours at 150mA at 4°C, in transferring buffer (0.3 % Tris base, 1.44 % glycine, 20% methanol). The membrane was saturated by overnight incubation at 4°C in saturation buffer (10% skimmed milk, 0.1% Triton X100 in PBS). The membrane was washed twice with washing buffer (3% skimmed milk, 0.1% Triton X100 in PBS) and incubated for 2 hours at 37°C with 1:200 mice sera diluted in washing buffer. The membrane was washed twice and incubated for 90 minutes with a 1:2000 dilution of horseradish peroxidase labeled anti-mouse Ig. The membrane was washed twice with 0.1% Triton X100 in PBS and developed with the Opti-4CN Substrate Kit (Bio-Rad). The reaction was stopped by adding water.

Bactericidal assay

MC58 strain was grown overnight at 37°C on chocolate agar plates. 5-7 colonies were collected and used to inoculate 7ml Mueller-Hintón broth. The suspension was incubated at 37°C on a nutator and let to grow until OD₆₂₀ was in between 0.5-0.8. The culture was aliquoted into sterile 1.5ml Eppendorf tubes and centrifuged for 20 minutes at maximum speed in a microfuge. The pellet was washed once in Gey's buffer (Gibco) and resuspended in the same buffer to an OD₆₂₀ of 0.5, diluted 1:20000 in Gey's buffer and stored at 25°C.

50µl of Gey's buffer/1% BSA was added to each well of a 96-well tissue culture plate. 25µl of diluted (1:100) mice sera (dilution buffer: Gey's buffer/0.2% BSA) were added to each well and the plate incubated at 4°C. 25µl of the previously described bacterial suspension were added to each well. 25µl of either heat-inactivated (56°C waterbath for 30 minutes) or normal baby rabbit complement were added to each well. Immediately after the addition of the baby rabbit complement, 22µl of each sample/well were plated on Mueller-

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Hinton agar plates (time 0). The 96-well plate was incubated for 1 hour at 37°C with rotation and then 22µl of each sample/well were plated on Mueller-Hinton agar plates (time 1). After overnight incubation the colonies corresponding to time 0 and time 1h were counted.

The following DNA and amino acid sequences are identified by titles of the following form: [g, m, or a] [#].[seq or pep], where "g" means a sequence from *N. gonorrhoeae*, "m" means a sequence from *N. meningitidis B*, and "a" means a sequence from *N. meningitidis A*; "#" means the number of the sequence; "seq" means a DNA sequence, and "pep" means an amino acid sequence. For example, "g001.seq" refers to an *N. gonorrhoeae* DNA sequence, number 1. The presence of the suffix "-1" or "-2" to these sequences indicates an additional sequence found for the same ORF. Further, open reading frames are identified as ORF #, where "#" means the number of the ORF, corresponding to the number of the sequence which encodes the ORF, and the ORF designations may be suffixed with ".ng" or ".a", indicating that the ORF corresponds to a *N. gonorrhoeae* sequence or a *N. meningitidis A* sequence, respectively. Computer analysis was performed for the comparisons that follow between "g", "m", and "a" peptide sequences; and therein the "pep" suffix is implied where not expressly stated.

EXAMPLE 1

The following ORFs were predicted from the contig sequences and/or the full length sequences using the methods herein described.

Localization of the ORFs

ORF: contig:

279 gnm4.seq

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 2>:
m279.seq

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1  ATAACGCGGA TTTGCGGCTG CTTGATTCA ACGGTTTTCA GGGCTTCGGC
51  AAGTTTGTCT GCGGCGGGTT TCATCAGGCT GCAATGGGAA GGTACGGACA
101 CGGGCAGCGG CAGGCGCGGT TTGGCACCGG CTTCTTTGGC GGCAGCCATG
151 GCGCGTCCGA CGGCGGCGGC GTTGCCCTGCA ATCACGATT GTCCGGGTGA
201 GTTGAAGTTG ACGGCTTCGA CCACTTCGCT TTGGGCGGCT TCGGCACAAA
251 TGGCTTTTAA CTGCTCATCT TCCAAGCCGA GAATCGCCGC CATTGCGCCC
301 ACGCCTTGCG GTACGGCGGA CTGCATCAGT TCGGCGCGCA GGCGCACGAG
351 TTGACCGCG TCGGCAAAAT TCAATGCGCC GCGGCAACG AGTGCGGTGT
401 ATTCGCCGAG GCTGTGTCCG GCAACGGCGG CAGGCGTTTT GCCGCCGCT
451 TCTAAATAG

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401 ATTCGCCGAN GCTGTGTCCG GCAACGGCGG CAGGCGTTTT GCCGCCGCT
451 TCCGAATAG

This corresponds to the amino acid sequence <SEQ ID 7; ORF 279.a>:

a279.pep
1 MTXICGCLIS TVXRASASLS AAGFMRLQWE GTDTGSGRAR LAPASLAASI
51 ARSTAAALPA ITTCPGELKL TASTTSSCAD SAQICFTCSS SKPRIAAIAP
101 TPCGTADCIS SARXRTSLTA SAKSNAPAAT SAVYSPXLCP ATAAGVLPFA
151 SE*

m279/a279 ORFs 279 and 279.a showed a 88.2% identity in 152 aa overlap

	10	20	30	40	50	60
m279.pep	ITRICGCLISTVFRASASLSAAGFIRLQWEGTDTGSGRARLAPASLAAMARPTAAALPA					
a279	MTXICGCLISTVXRASASLSAAGFMRLQWEGTDTGSGRARLAPASLAASIARSTAAALPA					
	10	20	30	40	50	60
	70	80	90	100	110	120
m279.pep	ITICPGELKLTAATTSWAASAQMALTCSSSKPRIAAIAPTPCGTADCISSARRRTSLTA					
a279	ITTCPGELKLTAATTSWADSAQICFTCSSSKPRIAAIAPTPCGTADCISSARXRTSLTA					
	70	80	90	100	110	120
	130	140	150			
m279.pep	SAKFNAPAATSAVYSPRLCPATAAGVLPPASKX					
a279	SAKSNAPAATSAVYSPXLCPATAAGVLPPASEX					
	130	140	150			

519 and 519-1 gnm7.seq

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 8>:

m519.seq (partial)
1 ..TCCGTTATCG GCGGTATGGA GTTGGACAAA ACGTTTGAAG AACGCGACGA
51 AATCAACAGT ACTGTTGTTG CGGCTTTGGA CGAGGCGGCC GGGgCTTgGG
101 GTGTGAAGGT TTTGCGTTAT GAGATTAAAG ACTTGGTTCC GCCGCAAGAA
151 ATCCTTCGCT CAATGCAGGC GCAAATTACT GCCGAACGCG AAAAAACGCGC
201 CCGTATCGCC GAATCCGAAG GTCGTAAAAT CGAACAAATC AACCTTGCCA
251 GTGGTCAGCG CGAAGCCGAA ATCCAACAAT CCGAAGGCGA GGCTCAGGCT
301 GCGGTCAATG CGTCAAATGC CGAGAAAATC GCCCGCATCA ACCGCGCCAA
351 AGGTGAAGCG GAATCCTTGC GCCTTGTTGC CGAAGCCAAT GCCGAAGCCA
401 TCCGTCAAAT TGCCGCCGCC CTTCAAACCC AAGGCGGTGC GGATGCGGTC
451 AATCTGAAGA TTGCGGAACA ATACGTCGCT GCGTCAACA ATCTTGCCAA
501 AGAAAGCAAT ACGCTGATTA TGCCCGCCAA TGTTGCCGAC ATCGGCAGCC
551 TGATTTCTGC CGGTATGAAA ATTATCGACA GCAGCAAAAC CGCCAAaTAA

This corresponds to the amino acid sequence <SEQ ID 9; ORF 519>:

m519.pep (partial)
1 ..SVIGRMELDK TFEERDEINS TVVAALDEAA GAWGVKVLRY EIKDLVPPQE
51 ILRSMQAQIT AEREKRARIA ESEGRKIEQI NLASGQREAE IQQSEGEAQA
101 AVNASNAEKI ARINRAKGEA ESLRLVAEAN AEAIQIAAAA LQTQGGADAV
151 NLKIAEQYVA AFNNLAKESN TLIMPANVAD IGLISAGMK IIDSSKTAK*

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 10>:

g519.seq
1 atggaatttt tcattatctt gttggcagcc gtcgcccgttt tcgggttcaa
51 atcctttgtc gtcaccccc agcaggaagt ccacgttgtc gaaaggtcgc

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101 ggcgtttcca tcgcgccttg acggccggtt tgaatatattt gattcccttt
151 atcgaccgcy tcgcctaccg ccattcgctg aaagaaatcc ctttagacgt
201 acccagccag gtctgcatca cgcgcgataa tacgcaattg actgttgacg
251 gcatcatcta tttccaagta accgatccca aactcgcctc atacggttcg
301 agcaactaca ttatggcaat taccagctt gcccaaacga cgctcggttc
351 cgttatcggy cgtatggagt tggacaaaac gtttgaagaa cgcgacgaaa
401 tcaacagtac cgtcgctctc gccctcgatg aagccgcggy ggcttggggt
451 gtgaaagtcc tccgttacga aatcaaggat ttggttccgc cgcaagaaat
501 ccttcgcyca atgcaggcac aaattaccgc cgaacgcgaa aaacgcgccc
551 gtattgcyga atccgaaggc cgtaaaatcg aacaaatcaa ccttgccagt
601 ggtcagcgtg aagccgaaat ccaacaatcc gaaggcgagg cttaggctgc
651 ggtcaatgcy tccaatgccg agaaaatcgc ccgcatcaac cgcgccaag
701 gcgaagcgga atccctgcgc cttgttgccg aagccaatgc cgaagccaac
751 cgtcaaattg ccgccgccct tcaaacccaa agcggggcgy atgcggtcaa
801 tctgaagatt ccgggacaat acgttaccgc gttcaaaaat cttgccaag
851 aagacaatac gcggattaag cccgccaagg ttgccgaaat cgggaaccct
901 aattttcgcy ggcatgaaaa attttcgcca gaagcaaaaa cggccaata
951 a

```

This corresponds to the amino acid sequence <SEQ ID 11; ORF 519.ng>:

```

g519.pep
1  MEFFIILLAA VAVFGFKSFV VIPQEVHV V ERLGRFHRAL TAGLNILIPF
51  IDRVAYRHS L KEIPLDVPSQ VCITRDNTQL TVDGIIYFQV TDPKLASYGS
101 SNYIMAITQL AQTLRSVIG RMELDKTFEE RDEINSTVVS ALDEAAGAWG
151 VKVLRYEIKD LVPPQEILRA MQAQITAERE KRARIAESEG RKIEQINLAS
201 GQREAEIQQS EGEAQA AVNA SNAEKIARIN RAKGEAESLR LVAEANA EAN
251 RQIAAALQTO SGADAVNLKI AGQYVTA FKN LAKEDNTRIK PAKVAEIGNP
301 NFRRHEKFSP EAKTAK*

```

ORF 519 shows 87.5% identity over a 200 aa overlap with a predicted ORF (ORF 519.ng) from *N. gonorrhoeae*:

m519/g519

```

m519.pep
SVIGRMELDKTFEERDEINSTVVAALDEAA
g519
YFQVTDPKLASYGSSNYIMAITQLAQTTLSVIGRMELDKTFEERDEINSTVVSALDEAA
90 100 110 120 130 140

m519.pep
GAWGVKVLRYEIKDLVPPQEILRSMQQAQITAEREKRARIAESEGRKIEQINLASGQREAE
g519
GAWGVKVLRYEIKDLVPPQEILRAMQAQITAEREKRARIAESEGRKIEQINLASGQREAE
150 160 170 180 190 200

m519.pep
IQQSEGEAQA AVNASNAEKIARINRAKGEAESLRLVAEANA EAIRQIAAALQTGGADAV
g519
IQQSEGEAQA AVNASNAEKIARINRAKGEAESLRLVAEANA EANRQIAAALQTQSGADAV
210 220 230 240 250 260

m519.pep
NLKIAEQYVA AFNNLAKESNTLIMPANVADIGSL- ISAGMKIIDSSKTAK
g519
NLKIAEQYVTA FKNLAKEDNTRIKPAKVAEIGNPNFRRHEKFSP EAKTAK
270 280 290 300 310

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 12>:

a519.seq

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```

1 ATGGAATTTT TCATTATCTT GCTGGCAGCC GTCGTTGTTT TCGGCTTCAA
51 ATCCTTTGTT GTCATCCCAC AGCAGGAAGT CCACGTTGTC GAAAGGCTCG
101 GCGGTTTCCA TCGCGCCCTG ACGGCCGGTT TGAATATTTT GATTCCCTTT
151 ATCGACCGCG TCGCCTACCG CCATTGCTG AAAGAAATCC CTTTAGACGT
201 ACCCAGCCAG GTCTGCATCA CGCGCGACAA TACGCAGCTG ACTGTTGACG
251 GTATCATCTA TTCCAAGTA ACCGACCCCA AACTCGCCTC ATACGGTTTCG
301 AGCAACTACA TTATGGCGAT TACCCAGCTT GCCCAAACGA CGCTGCGTTC
351 CGTTATCGGG CGTATGGAAT TGGACAAAAC GTTTGAAGAA CGCGACGAAA
401 TCAACAGCAC CGTCGTCTCC GCCCTCGATG AAGCCGCCGG AGCTTGGGGT
451 GTGAAGGTTT TGCCTTATGA GATTAAAGAC TTGGTTCCGC CGCAAGAAAT
501 CCTTCGCTCA ATGCAGGCGC AAATTACTGC TGAACGCGAA AAACGCGCCC
551 GTATCGCCGA ATCCGAAGGT CGTAAAATCG AACAAATCAA CCTTGCCAGT
601 GGTCAAGCGG AAGCCGAAAT CCAACAATCC GAAGCGGAGG CTCAGGCTGC
651 GGTCAATGCG TCAAATGCCG AGAAAATCGC CCGCATCAAC CGCGCCAAAG
701 GTGAAGCGGA ATCCTTGCGC CTTGTTGCCG AAGCCAATGC CGAAGCCATC
751 CGTCAAATTG CCGCCGCCCT TCAAACCCAA GGCGGTGCGG ATGCGGTCAA
801 TCTGAAGATT GCGGAACAAT ACGTCGCCGC GTTCAACAAT CTTGCCAAAG
851 AAAGCAATAC GCTGATTATG CCCGCCAATG TTGCCGACAT CGGAGCCTG
901 ATTTCTGCCG GTATGAAAAT TATCGACAGC AGCAAAACCG CCAATAAA

```

This corresponds to the amino acid sequence <SEQ ID 13; ORF 519.a>:

```

a519.pep
1 MEFFIILLAA VVVFQKSFV VIPQEVHV V ERLGRFHRAL TAGLNILIPF
51 IDRVAYRHSI KEIPLDVPSQ VCITRDNTQL TVDGIIYFQV TDPKLASYGS
101 SNYIMAITQL AQTTLRSVIG RMELDKTFEE RDEINSTVVS ALDEAAGAWG
151 VKVLRYEIKD LVPPQEILRS MQAQITAERE KRARIAESEG RKIEQINLAS
201 GOREAEIQQS EGEAQAAVNA SNAEKIARIN RAKGEAESLR LVAEANAIAI
251 RQIAAALQTO GGADAVNLKI AEQYVAAFNN LAKESNTLIM PANVADIGSL
301 ISAGMKIIDS SKTAK*

m519/a519 ORFs 519 and 519.a showed a 99.5% identity in 199 aa overlap

m519.pep
10 20 30
SVIGRMELDKTFEERDEINSTVVAALDEAA
|||||:|||||
a519 YFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIGRMELDKTFEERDEINSTVVSALDEAA
90 100 110 120 130 140

m519.pep
40 50 60 70 80 90
GAWGVKVLRYEIKDLVPPQEILRSMAQITAEREKRARIAESEGRKIEQINLASGQREAE
|||||:|||||
a519 GAWGVKVLRYEIKDLVPPQEILRSMAQITAEREKRARIAESEGRKIEQINLASGQREAE
150 160 170 180 190 200

m519.pep
100 110 120 130 140 150
IQQSEGEAQAAVNASNAEKIARINRAKGEAESLRLVAEANAIAIRQIAAALQTGGADAV
|||||:|||||
a519 IQQSEGEAQAAVNASNAEKIARINRAKGEAESLRLVAEANAIAIRQIAAALQTGGADAV
210 220 230 240 250 260

m519.pep
160 170 180 190 200
NLKIAEQYVAAFNNLAKESNTLIMPANVADIGSLISAGMKIIDSSKTAKX
|||||:|||||
a519 NLKIAEQYVAAFNNLAKESNTLIMPANVADIGSLISAGMKIIDSSKTAKX
270 280 290 300 310

```

Further work revealed the following DNA sequence identified in *N. meningitidis* <SEQ ID 14>:

m519-1.seq

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```

1  ATGGAATTTT TCATTATCTT GTTGGTAGCC GTCGCCGTTT TCGGTTTCAA
51  ATCCTTTGTT GTCATCCAC AACAGGAAGT CCACGTTGTC GAAAGGCTGG
101 GCGGTTTCCA TCGCGCCCTG ACGGCGGTT TGAATATTTT GATTCCCTTT
151 ATCGACCGCG TCGCCTACCG CCATTGCGTG AAAGAAATCC CTTTAGACGT
201 ACCAGCCAG GTCTGCATCA CGCGCGACAA TACGCAGCTG ACTGTTGACG
251 GCATCATCTA TTTCCAAGTA ACCGACCCCA AACTCGCCTC ATACGGTTCCG
301 AGCAACTACA TTATGGCGAT TACCCAGCTT GCCCAAACGA CGCTGCGTTC
351 CGTTATCGGG CGTATGGAGT TGGACAAAAC GTTTGAAGAA CGCGACGAAA
401 TCAACAGTAC TGTTGTTGCG GCTTTGGACG AGGCGGCCGG GGCTTGGGGT
451 GTGAAGGTTT TCGGTTATGA GATTAAAGAC TTGGTTCCGC CGCAAGAAAT
501 CCTTCGCTCA ATGCAGGCGC AAATTACTGC CGAACGCGAA AAACGCGCCC
551 GTATCGCCGA ATCCGAAGGT CGTAAAATCG AACAAATCAA CCTTGCCAGT
601 GGTCAAGCGG AAGCCGAAAT CCAACAATCC GAAGGCGAGG CTCAGGCTGC
651 GGTCAATGCG TCAAATGCCG AGAAAATCGC CCGCATCAAC CGCGCCAAAG
701 GTGAAGCGGA ATCCTTGCGC CTGTGTGCGG AAGCCAATGC CGAAGCCATC
751 CGTCAAATTG CCGCGGCCCT TCAAACCCAA GGCGGTGCGG ATGCGGTCAA
801 TCTGAAGATT GCGGAACAAT ACGTCGCTGC GTTCAACAAT CTTGCCAAAG
851 AAAGCAATAC GCTGATTATG CCCGCCAATG TTGCCGACAT CGGCAGCCTG
901 ATTTCTGCCG GTATGAAAAT TATCGACAGC AGCAAAACCG CCAAATAA

```

This corresponds to the amino acid sequence <SEQ ID 15; ORF 519-1>:

m519-1.

```

1  MEFFIILLVA VAVFGFKSFV VIPQOEHVHV ERLGRFHRAL TAGLNILIPF
51  IDRVAYRHSI KEIPLDVPSQ VCITRDNTQL TVDGIIFYQV TDPKLASYGS
101 SNYIMAITQL AQTTLRSVIG RMELDKTFEE RDEINSTVVA ALDEAAGAWG
151 VKVLRYEIKD LVPPQEILRS MQAQITAERE KRARIAESEG RKIEQINLAS
201 GQREAEIQQS EGEEAQAQVNA SNAEKIARIN RAKGEAESLR LVAEANAIAI
251 RQIAAALQTQ GGADAVNLKI AEQYVAAFNN LAKESNTLIM PANVADIGSL
301 ISAGMKIIDS SKTAK*

```

The following DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 16>:

g519-1.seq

```

1  ATGGAATTTT TCATTATCTT GTTGGCAGCC GTCGCCGTTT TCGGCTTCAA
51  ATCCTTTGTC GTCATCCCCC AGCAGGAAGT CCACGTTGTC GAAAGGCTCG
101 GCGGTTTCCA TCGCGCCCTG ACGGCGGTT TGAATATTTT GATTCCCTTT
151 ATCGACCGCG TCGCCTACCG CCATTGCGTG AAAGAAATCC CTTTAGACGT
201 ACCAGCCAG GTCTGCATCA CGCGCGATAA TACGCAATTG ACTGTTGACG
251 GCATCATCTA TTTCCAAGTA ACCGATCCCA AACTCGCCTC ATACGGTTCCG
301 AGCAACTACA TTATGGCAAT TACCCAGCTT GCCCAAACGA CGCTGCGTTC
351 CGTTATCGGG CGTATGGAGT TGGACAAAAC GTTTGAAGAA CGCGACGAAA
401 TCAACAGTAC CGTCGTCTCC GCCCTCGATG AAGCCGCCGG GGCTTGGGGT
451 GTGAAAGTCC TCCGTTACGA AATCAAGGAT TTGGTTCCGC CGCAAGAAAT
501 CCTTCGCGCA ATGCAGGCAC AAATTACCGC CGAACGCGAA AAACGCGCCC
551 GTATTGCCGA ATCCGAAGGC CGTAAAATCG AACAAATCAA CCTTGCCAGT
601 GGTCAAGCTG AAGCCGAAAT CCAACAATCC GAAGGCGAGG CTCAGGCTGC
651 GGTCAATGCG TCCAATGCCG AGAAAATCGC CCGCATCAAC CGCGCCAAAG
701 GCGAAGCGGA ATCCTTGCGC CTGTGTGCGG AAGCCAATGC CGAAGCCATC
751 CGTCAAATTG CCGCGGCCCT TCAAACCCAA GGCGGGGCGG ATGCGGTCAA
801 TCTGAAGATT GCGGAACAAT ACGTAGCCGC GTTCAACAAT CTTGCCAAAG
851 AAAGCAATAC GCTGATTATG CCCGCCAATG TTGCCGACAT CGGCAGCCTG
901 ATTTCTGCCG GCATGAAAAT TATCGACAGC AGCAAAACCG CCAAATAA

```

This corresponds to the amino acid sequence <SEQ ID 17; ORF 519-1.ng>:

g519-1.pep

```

1  MEFFIILLAA VAVFGFKSFV VIPQOEHVHV ERLGRFHRAL TAGLNILIPF
51  IDRVAYRHSI KEIPLDVPSQ VCITRDNTQL TVDGIIFYQV TDPKLASYGS
101 SNYIMAITQL AQTTLRSVIG RMELDKTFEE RDEINSTVVS ALDEAAGAWG
151 VKVLRYEIKD LVPPQEILRA MQAQITAERE KRARIAESEG RKIEQINLAS
201 GQREAEIQQS EGEEAQAQVNA SNAEKIARIN RAKGEAESLR LVAEANAIAI
251 RQIAAALQTQ GGADAVNLKI AEQYVAAFNN LAKESNTLIM PANVADIGSL
301 ISAGMKIIDS SKTAK*

```

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m519-1/g519-1 ORFs 519-1 and 519-1.ng showed a 99.0% identity in 315 aa overlap

	10	20	30	40	50	60
g519-1.pep	MEFFIILLAAVAVFGFKSFVVIPQQEVHVVERLGRFHRALTAGLNILIPFIDRVAYRHSL					
m519-1	MEFFIILLVAVAVFGFKSFVVIPQQEVHVVERLGRFHRALTAGLNILIPFIDRVAYRHSL					
	10	20	30	40	50	60
	70	80	90	100	110	120
g519-1.pep	KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG					
m519-1	KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG					
	70	80	90	100	110	120
	130	140	150	160	170	180
g519-1.pep	RMELDKTFEERDEINSTVVSALDEAAGAWGVKVLRYEIKDLVPPQEILRAMQAQITAERE					
m519-1	RMELDKTFEERDEINSTVVAALDEAAGAWGVKVLRYEIKDLVPPQEILRSMQAQITAERE					
	130	140	150	160	170	180
	190	200	210	220	230	240
g519-1.pep	KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR					
m519-1	KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR					
	190	200	210	220	230	240
	250	260	270	280	290	300
g519-1.pep	LVAEANAIAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL					
m519-1	LVAEANAIAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL					
	250	260	270	280	290	300
	310					
g519-1.pep	ISAGMKIIDSSKTAKX					
m519-1	ISAGMKIIDSSKTAKX					
	310					

The following DNA sequence was identified in *N. meningitidis* <SEQ ID 18>:

a519-1.seq

1	ATGGAATTTT	TCATTATCTT	GCTGGCAGCC	GTCTGTGTTT	TCGGCTTCAA
51	ATCCTTTGTT	GTCATCCAC	AGCAGGAAGT	CCACGTTGTC	GAAAGGCTCG
101	GGCGTTTCCA	TCGCGCCCTG	ACGGCCGTT	TGAATATTTT	GATTCCCTTT
151	ATCGACCGCG	TCGCCTACCG	CCATTGCTG	AAAGAAATCC	CTTTAGACGT
201	ACCCAGCCAG	GTCTGCATCA	CGCGCGACAA	TACGCAGCTG	ACTGTTGACG
251	GTATCATCTA	TTTCCAAGTA	ACCGACCCCA	AACTCGCCTC	ATACGTTTCG
301	AGCAACTACA	TTATGGCGAT	TACCCAGCTT	GCCCAAACGA	CGCTGCGTTC
351	CGTTATCGGG	CGTATGGAAT	TGGACAAAAC	GTTTGAAGAA	CGCGACGAAA
401	TCAACAGCAC	CGTCGTCTCC	GCCCTCGATG	AAGCCGCCGG	AGCTTGGGGT
451	GTGAAGGTTT	TGCGTTATGA	GATTAAAGAC	TTGGTTCCGC	CGCAAGAAAT
501	CCTTCGCTCA	ATGCAGGCGC	AAATTACTGC	TGAACGCGAA	AAACGCGCCC
551	GTATCGCCGA	ATCCGAAGGT	CGTAAATCG	AACAAATCAA	CCTTGCCAGT
601	GGTCAGCGCG	AAGCCGAAAT	CCAACAATCC	GAAGGCGAGG	CTCAGGCTGC
651	GGTCAATGCG	TCAAATGCCG	AGAAAATCGC	CCGCATCAAC	CGCGCCAAAG
701	GTGAAGCGGA	ATCCTTGCGC	CTTGTTGCCG	AAGCCAATGC	CGAAGCCATC
751	CGTCAAATTG	CCGCCGCCCT	TCAAACCCAA	GGCGGTGCGG	ATGCGGTCAA
801	TCTGAAGATT	GCGGAACAAT	ACGTCGCCGC	GTTCAACAAT	CCTTGCCAAAG
851	AAAGCAATAC	GCTGATTATG	CCCGCCAATG	TTGCCGACAT	CGGCAGCCTG
901	ATTTCTGCCG	GTATGAAAAT	TATCGACAGC	AGCAAAACCG	CCAAATAA

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This corresponds to the amino acid sequence <SEQ ID 19; ORF 519-1.a>:

a519-1.pep.

```

1  MEFFIILLAA VVVFGEKSFV VIPQEVHV V ERLGRFHRAL TAGLNILIPF
51  IDRVAYRHSL KEIPLDVPSQ VCITRDNTQL TVDGIIFYQV TDPKLASYGS
101 SNYIMAITQL AQTTLRSVIG RMELDKTFEE RDEINSTVVS ALDEAAGAWG
151 VKVLRYEIKD LVPPQEILRS MQAQITAERE KRARIAESEG RKIEQINLAS
201 QREAEIQQS EGEAQAAVNA SNAEKIARIN RAKGEAESLR LVAEANAIAI
251 RQIAAALQTQ GGADAVNLKI AEQYVAAFNN LAKESNTLIM PANVADIGSL
301 ISAGMKIIDS SKTAK*

```

m519-1/a519-1 ORFs 519-1 and 519-1.a showed a 99.0% identity in 315 aa overlap

	10	20	30	40	50	60
a519-1.pep	MEFFIILLAAVVVFGEKSFV	VIPQEVHVVERLGRFHRAL	TAGLNILIPFIDRVAYRHSL			
m519-1	MEFFIILLVAVAVFGKSFV	VIPQEVHVVERLGRFHRAL	TAGLNILIPFIDRVAYRHSL			
	10	20	30	40	50	60
a519-1.pep	KEIPLDVPSQVCITRDNTQL	TVDGIIFYQVTDPKLASYGSS	SNYIMAITQLAQTTLRSVIG			
m519-1	KEIPLDVPSQVCITRDNTQL	TVDGIIFYQVTDPKLASYGSS	SNYIMAITQLAQTTLRSVIG			
	70	80	90	100	110	120
a519-1.pep	KEIPLDVPSQVCITRDNTQL	TVDGIIFYQVTDPKLASYGSS	SNYIMAITQLAQTTLRSVIG			
m519-1	KEIPLDVPSQVCITRDNTQL	TVDGIIFYQVTDPKLASYGSS	SNYIMAITQLAQTTLRSVIG			
	70	80	90	100	110	120
a519-1.pep	RMELDKTFEERDEINSTVVS	ALDEAAGAWGVKVLRYEIKD	LVPPQEILRSMQQAITAERE			
m519-1	RMELDKTFEERDEINSTVVA	ALDEAAGAWGVKVLRYEIKD	LVPPQEILRSMQQAITAERE			
	130	140	150	160	170	180
a519-1.pep	RMELDKTFEERDEINSTVVS	ALDEAAGAWGVKVLRYEIKD	LVPPQEILRSMQQAITAERE			
m519-1	RMELDKTFEERDEINSTVVA	ALDEAAGAWGVKVLRYEIKD	LVPPQEILRSMQQAITAERE			
	130	140	150	160	170	180
a519-1.pep	KRARIAESEGRKIEQINLAS	GQREAEIQQSEGEAQAAVNA	SNAEKIARINRAKGEAESLR			
m519-1	KRARIAESEGRKIEQINLAS	GQREAEIQQSEGEAQAAVNA	SNAEKIARINRAKGEAESLR			
	190	200	210	220	230	240
a519-1.pep	KRARIAESEGRKIEQINLAS	GQREAEIQQSEGEAQAAVNA	SNAEKIARINRAKGEAESLR			
m519-1	KRARIAESEGRKIEQINLAS	GQREAEIQQSEGEAQAAVNA	SNAEKIARINRAKGEAESLR			
	190	200	210	220	230	240
a519-1.pep	LVAEANAIAIRQIAAALQTQ	GGADAVNLKIAEQYVAAFNN	LAKESNTLIMPANVADIGSL			
m519-1	LVAEANAIAIRQIAAALQTQ	GGADAVNLKIAEQYVAAFNN	LAKESNTLIMPANVADIGSL			
	250	260	270	280	290	300
a519-1.pep	LVAEANAIAIRQIAAALQTQ	GGADAVNLKIAEQYVAAFNN	LAKESNTLIMPANVADIGSL			
m519-1	LVAEANAIAIRQIAAALQTQ	GGADAVNLKIAEQYVAAFNN	LAKESNTLIMPANVADIGSL			
	250	260	270	280	290	300
a519-1.pep	ISAGMKIIDSSKTAKX					
m519-1	ISAGMKIIDSSKTAKX					
	310					

576 and 576-1 gnm22.seq

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 20>:

m576.seq.. (partial)

```

1  ..ATGCAGCAGG CAAGCTATGC GATGGGCGTG GACATCGGAC GCTCCCTGAA
51  GCAATGAAG GAACAGGGCG CGGAAATCGA TTGAAAGTC TTTACCGAAG
101 CCATGCAGGC AGTGTATGAC GGCAAAGAAA TCAAATGAC CGAAGAGCAG
151 GCTCAGGAAG TCATGATGAA ATTCCTCAG GAACAACAGG CTAAAGCCGT
201 AGAAAAACAC AAGGCGGACG CGAAGGCCAA TAAAGAAAAA GGCGAAGCCT

```

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```

251 TTCTGAAAGA AAATGCCGCC AAAGACGGCG TGAAGACCAC TGCTTCCGGC
301 CTGCAATACA AAATCACCAA ACAGGGCGAA GGCAAACAGC CGACCAAAGA
351 CGACATCGTT ACCGTGGAAT ACGAAGGCCG CCTGATTGAC GGTACGGTAT
401 TCGACAGCAG CAAAGCCAAC GCGGCCCGG TCACCTTCCC TTTGAGCCAA
451 GTGATTCCGG GTTGGACCGA AGCGGTACAG CTTCTGAAAG AAGGCGGCGA
501 AGCCACGTTC TACATCCCCT CCAACCTTGC CTACCGCGAA CAGGGTCCGG
551 GCGACAAAAT CCGTCCGAAC GCCACTTTGG TATTTGATGT GAAACTGGTC
601 AAAATCGGCG CACCCGAAAA CGCGCCCGCC AAGCAGCCGG CTCAAGTCGA
651 CATCAAAAAA GTAAATTAA

```

This corresponds to the amino acid sequence <SEQ ID 21; ORF 576>:

```

m576.pep.. (partial)
  1 ..MQQASYAMGV DIGRSLKQMK EQGAEIDLKV FTEAMQAVYD GKEIKMTEEO
 51 AQEVMKFLQ EQQAKAVEKH KADAKANKEK GEAFLENAA KDGVKTTASG
101 LQYKITKQGE GKQPTKDDIV TVEYEGRLID GTVFDSSKAN GGPVTFPLSQ
151 VIPGWTEGVQ LLKEGGEATF YIPSNLAYRE QGAGDKIGPN ATLVDVVKLV
201 KIGAPENAPA KQPAQVDIKK VN*

```

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 22>:

```

g576.seq.. (partial)
  1 ..atgggcggtgg acatcggacg ctcctgaaa caaatgaagg aacagggcgc
 51 ggaaatcgat ttgaaagtct ttaccgatgc catgcaggca gtgtatgacg
101 gcaaagaaat caaatgacc gaagagcagg cccagggaagt gatgatgaaa
151 ttcctgcagg agcagcaggc taaagccgta gaaaaacaca aggcggatgc
201 gaaggccaac aaagaaaaag gcgaagcctt cctgaaggaa aatgccgcgc
251 aagacggcgt gaagaccact gcttcgggtc tgcagtacaa aatcaccaaa
301 cagggtgaag gcaaacagcc gacaaaagac gacatcgtaa ccgtggaata
351 cgaaggccgc ctgattgacg gtaccgtatt cgacagcagc aaagccaacg
401 gcggcccggc cacccttcct ttgagccaag tgattccggg ttggaccgaa
451 ggcgtacggc ttctgaaaga aggcggcgaa gccacgttct acatcccgtc
501 caaccttgcc taccgcgaac aggggtgcgg cgaaaaaatc ggtccgaacg
551 ccactttggt atttgacgtg aaactgggtc aaatcggcgc acccgaaaac
601 gcgcccgcga agcagccgga tcaagtcgac atcaaaaaag taaattaa

```

This corresponds to the amino acid sequence <SEQ ID 23; ORF 576.ng>:

```

g576.pep.. (partial)
  1 ..MGVDIGRSLK QMKEQGAEID LKVFTDAMQA VYDGKEIKMT EEQAQEVMMK
 51 FLQEQQAKAV EKHKADAKAN KEKGEAFLKE NAAEDGVKTT ASGLQYKITK
101 QGEGKQPTKD DIVTVEYEGR LIDGTVFDSS KANGGPATFP LSQVIPGWTE
151 GVRLLKEGGE ATFYIPSNLA YREQGAGEKI GPNATLVFDV KLVKIGAPEN
201 APAKQPDQVD IKKVN*

```

Computer analysis of this amino acid sequence gave the following results:

Homology with a predicted ORF from *N. gonorrhoeae*

m576/g576 97.2% identity in 215 aa overlap

```

              10      20      30      40      50      60
m576.pep      MQQASYAMGV DIGRSLKQMK EQGAEIDLKV FTEAMQAVYDGKEIKMTEEQAEVMMKFLQ
              |||
g576           MGVDIGRSLKQMK EQGAEIDLKV FTEAMQAVYDGKEIKMTEEQAEVMMKFLQ
              10      20      30      40      50

              70      80      90     100     110     120
m576.pep      EQQAKAVEKH KADAKANKEK GEAFLENAA KDGVKTTASGLQYKITKQGEKGQPTKDDIV
              |||
g576           EQQAKAVEKH KADAKANKEK GEAFLENAA KDGVKTTASGLQYKITKQGEKGQPTKDDIV
              60      70      80      90     100     110

```

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	130	140	150	160	170	180
m576.pep	TVEYEGRLIDGTVFDSSKANGGPVTFPLSQVIPGWTEGVQLLKEGGEATFYIPSNLAYRE					
	: :					
g576	TVEYEGRLIDGTVFDSSKANGGPATFPLSQVIPGWTEGVRLLEGEATFYIPSNLAYRE					
	120	130	140	150	160	170

	190	200	210	220
m576.pep	QGAGDKIGPNATLVFDVKLVKIGAPENAPAKQPAQVDIKKVN			
	: :			
g576	QGAGEKIGPNATLVFDVKLVKIGAPENAPAKQPDQVDIKKVN			
	180	190	200	210

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 24>:

a576.seq

1	ATGAACACCA	TTTTCAAAT	CAGCGCACTG	ACCCTTTCCG	CCGCTTTGGC
51	ACTTTCGCC	TGCGGCCAAA	AAGAAGCCGC	CCCCGCATCT	GCATCCGAAC
101	CTGCCGCCG	TTCTTCCGCG	CAGGGCGACA	CCTCTTCGAT	CGGCAGCACG
151	ATGCAGCAGG	CAAGCTATGC	GATGGGCGTG	GACATCGGAC	GCTCCCTGAA
201	GCAAAATGAAG	GAACAGGGCG	CGGAAATCGA	TTTGAAAGTC	TTTACCGAAG
251	CCATGCAGGC	AGTGTATGAC	GGCAAAGAAA	TCAAAATGAC	CGAAGAGCAG
301	GCTCAGGAAG	TCATGATGAA	ATTCCTTCAG	GAACAACAGG	CTAAAGCCGT
351	AGAAAAACAC	AAGGCGGACG	CGAAGGCCAA	TAAAGAAAAA	GGCGAAGCCT
401	TTCTGAAAGA	AAATGCCGCC	AAAGACGGCG	TGAAGACCAC	TGCTTCCGGC
451	CTGCAATACA	AAATCACCAA	ACAGGGCGAA	GGCAAACAGC	CGACAAAGA
501	CGACATCGTT	ACCGTGGAAT	ACGAAGGCCG	CCTGATTGAC	GGTACGGTAT
551	TCGACAGCAG	CAAAGCCAAC	GGCGGCCCGG	TCACCTTCCC	TTTGAGCCAA
601	GTGATTCTGG	GTTGGACCGA	AGGCGTACAG	CTTCTGAAAG	AAGGCGGCCA
651	AGCCACGTTC	TACATCCCGT	CCAACCTTGC	CTACCGCGAA	CAGGGTGCGG
701	GCGACAAAAT	CGGCCCGAAC	GCCACTTTGG	TATTTGATGT	GAAACTGGTC
751	AAAATCGGCG	CACCCGAAAA	CGCGCCCGCC	AAGCAGCCGG	CTCAAGTCGA
801	CATCAAAAAA	GTAAATTAA			

This corresponds to the amino acid sequence <SEQ ID 25; ORF 576.a>:

a576.pep

1	MNTIFKISAL	TLAALALSA	CGKKEAAPAS	ASEPAAASSA	QGDTSSIGST
51	MQQASYAMGV	DIGRSLKQMK	EQGAEIDLKV	FTEAMQAVYD	GKEIKMTEEQ
101	AQEVMMKFLQ	EQQAKAVEKH	KADAKANKEK	GEAFLKENAA	KDGVKTTASG
151	LQYKITKQGE	GKQPTKDDIV	TVEYEGRLID	GTVFDSSKAN	GGPVTFFPLSQ
201	VILGWTEGVQ	LLKEGGEATF	YIPSNLAYRE	QGAGDKIGPN	ATLVFDVKLV
251	KIGAPENAPA	KQPAQVDIKK	VN*		

m576/a576 ORFs 576 and 576.a showed a 99.5% identity in 222 aa overlap

			10	20	30
m576.pep			MQQASYAMGVDIGRSLKQMKEQGAEIDLKV		
a576	CGKKEAAPASASEPAAASSAQGDTSSIGSTM	MQQASYAMGVDIGRSLKQMKEQGAEIDLKV			
	30	40	50	60	70
					80

	40	50	60	70	80	90
m576.pep	FTEAMQAVYDGKEIKMTEEQAQEVMMKFLQEQQAKAVEKHKADAKANKEKGEAFLKENAA					
a576	FTEAMQAVYDGKEIKMTEEQAQEVMMKFLQEQQAKAVEKHKADAKANKEKGEAFLKENAA					
	90	100	110	120	130	140

	100	110	120	130	140	150
m576.pep	KDGVKTTASGLQYKITKQEGKQPTKDDIVTVEYEGRLIDGTVFDSSKANGGPVTFPLSQ					
a576	KDGVKTTASGLQYKITKQEGKQPTKDDIVTVEYEGRLIDGTVFDSSKANGGPVTFPLSQ					
	150	160	170	180	190	200

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	160	170	180	190	200	210
m576.pep	VIPGWTEGVQLKEGGEATFYIPSNLAYREQGAGDKIGPNATLVFDVKLVKIGAPENAPA					
a576	VILGWTEGVQLKEGGEATFYIPSNLAYREQGAGDKIGPNATLVFDVKLVKIGAPENAPA					
	210	220	230	240	250	260
	220					
m576.pep	KQPAQVDIKKVN					
a576	KQPAQVDIKKVN					
	270					

Further work revealed the following DNA sequence identified in *N. meningitidis* <SEQ ID 26>:

m576-1.seq

1	ATGAACACCA	TTTTCAAAAT	CAGCGCACTG	ACCCCTTTCCG	CCGCTTTGGC
51	ACTTTCCGCC	TGCGGCAAAA	AAGAAGCCGC	CCCCGCATCT	GCATCCGAAC
101	CTGCCGCCGC	TTCTTCCGCG	CAGGGCGACA	CCTCTTCGAT	CGGCAGCACG
151	ATGCAGCAGG	CAAGCTATGC	GATGGGCGTG	GACATCGGAC	GCTCCCTGAA
201	GCAAATGAAG	GAACAGGGCG	CGGAAATCGA	TTTGAAAGTC	TTTACCGAAG
251	CCATGCAGGC	AGTGTATGAC	GGCAAAGAAA	TCAAAATGAC	CGAAGAGCAG
301	GCTCAGGAAG	TCATGATGAA	ATTCTTTCAG	GAACAACAGG	CTAAAGCCGT
351	AGAAAAACAC	AAGGCGGACG	CGAAGGCCAA	TAAAGAAAAA	GGCGAAGCCT
401	TTCTGAAAGA	AAATGCCGCC	AAAGACGGCG	TGAAGACCAC	TGCTTCCGGC
451	CTGCAATACA	AAATCACCAA	ACAGGGCGAA	GGCAAACAGC	CGACCAAAGA
501	CGACATCGTT	ACCGTGGAAT	ACGAAGGCCG	CCTGATTGAC	GGTACGGTAT
551	TCGACAGCAG	CAAAGCCAAC	GGCGGCCCGG	TCACCTTCCC	TTTGAGCCAA
601	GTGATTCCGG	GTTGGACCGA	AGGCGTACAG	CTTCTGAAAG	AAGGCGGCGA
651	AGCCACGTTT	TACATCCCGT	CCAACCTTGC	CTACCGCGAA	CAGGGTGCGG
701	GCGACAAAAT	CGGTCCGAAC	GCCACTTTGG	TATTTGATGT	GAAACTGGTC
751	AAAATCGGCG	CACCCGAAAA	CGCGCCCGCC	AAGCAGCCGG	CTCAAGTCGA
801	CATCAAAAAA	GTAATTAA			

This corresponds to the amino acid sequence <SEQ ID 27; ORF 576-1>:

m576-1.pep

1	MNTIFKISAL	TLAALALSA	CGKKEAAPAS	ASEPAAASSA	QGDTSSIGST
51	MQQASYAMGV	DIGRSLKQMK	EQGAEIDLKV	FTEAMQAVYD	GKEIKMTEEQ
101	AQEVMMKFQ	EQQAKAVEKH	KADAKANKEK	GEAFLKENAA	KDGVKTTASG
151	LQYKITKQGE	GKQPTKDDIV	TVEYEGRLID	GTVPDSSKAN	GGPVTFFPLSQ
201	VIPGWTEGVQ	LLKEGGEATF	YIPSNLAYRE	QGAGDKIGPN	ATLVFDVKLV
251	KIGAPENAPA	KQPAQVDIKK	VN*		

The following DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 28>:

g576-1.seq

1	ATGAACACCA	TTTTCAAAAT	CAGCGCACTG	ACCCCTTTCCG	CCGCTTTGGC
51	ACTTTCCGCC	TGCGGCAAAA	AAGAAGCCGC	CCCCGCATCT	GCATCCGAAC
101	CTGCCGCCGC	TTCTGCCGCG	CAGGGCGACA	CCTCTTCAAT	CGGCAGCACG
151	ATGCAGCAGG	CAAGCTATGC	AATGGGCGTG	GACATCGGAC	GCTCCCTGAA
201	ACAAATGAAG	GAACAGGGCG	CGGAAATCGA	TTTGAAAGTC	TTTACCGATG
251	CCATGCAGGC	AGTGTATGAC	GGCAAAGAAA	TCAAAATGAC	CGAAGAGCAG
301	GCCCAGGAAG	TGATGATGAA	ATTCTTGCAG	GAGCAGCAGG	CTAAAGCCGT
351	AGAAAAACAC	AAGGCGGATG	CGAAGGCCAA	CAAAGAAAAA	GGCGAAGCCT
401	TCCTGAAGGA	AAATGCCGCC	AAAGACGGCG	TGAAGACCAC	TGCTTCCGGT
451	CTGCAGTACA	AAATCACCAA	ACAGGGTGAA	GGCAAACAGC	CGACAAAAGA
501	CGACATCGTT	ACCGTGGAAT	ACGAAGGCCG	CCTGATTGAC	GGTACCGTAT
551	TCGACAGCAG	CAAAGCCAAC	GGCGGCCCGG	CCACCTTCCC	TTTGAGCCAA
601	GTGATTCCGG	GTTGGACCGA	AGGCGTACGG	CTTCTGAAAG	AAGGCGGCGA
651	AGCCACGTTT	TACATCCCGT	CCAACCTTGC	CTACCGCGAA	CAGGGTGCGG
701	GCGAAAAAAT	CGGTCCGAAC	GCCACTTTGG	TATTTGACGT	GAAACTGGTC
751	AAAATCGGCG	CACCCGAAAA	CGCGCCCGCC	AAGCAGCCGG	ATCAAGTCGA

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801 CATCAAAAAA GTAAATTAA

This corresponds to the amino acid sequence <SEQ ID 29; ORF 576-1.ng>:

g576-1.pep
 1 MNTIFKISAL TLSAALALS CGKKEAAPAS ASEPAASAA QGDTSSIGST
 51 MQQASYAMGV DIGRSLKQMK EQGAEIDLKV FTDAMQAVYD GKEIKMTEEQ
 101 AQEVMMKFLQ EQQAKAVEKH KADAKANKEK GEAFLENAA KDGVKTTASG
 151 LQYKITKQGE GKOPTKDDIV TVEYEGRLID GTVFDSSKAN GGPATFPLSQ
 201 VIPGWTEGVR LLKEGGEATF YIPSNLAYRE QGAGEKIGPN ATLVFDVKLV
 251 KIGAPENAPA KQPDQVDIKK VN*

g576-1/m576-1 ORFs 576-1 and 576-1.ng showed a 97.8% identity in 272 aa overlap

g576-1.pep	10	20	30	40	50	60
	MNTIFKISAL TLSAALALSACGKKEAAPASASEPAAASAAQGDTSIGSTMQQASYAMGV					
m576-1	10	20	30	40	50	60
	MNTIFKISAL TLSAALALSACGKKEAAPASASEPAAASSAQGDTSIGSTMQQASYAMGV					
g576-1.pep	70	80	90	100	110	120
	DIGRSLKQMK EQGAEIDLKV FTDAMQAVYD GKEIKMTEEQAQEVMMKFLQEQQAKAVEKH					
m576-1	70	80	90	100	110	120
	DIGRSLKQMK EQGAEIDLKV FTEAMQAVYD GKEIKMTEEQAQEVMMKFLQEQQAKAVEKH					
g576-1.pep	130	140	150	160	170	180
	KADAKANKEKGEAFLENAAKDGVKTTASGLQYKITKQGEKGKOPTKDDIVTVEYEGRLID					
m576-1	130	140	150	160	170	180
	KADAKANKEKGEAFLENAAKDGVKTTASGLQYKITKQGEKGKOPTKDDIVTVEYEGRLID					
g576-1.pep	190	200	210	220	230	240
	GTVFDSSKANGGPATFPLSQVIPGWTEGVRLLKEGGEATFYIPSNLAYREQGAGEKIGPN					
m576-1	190	200	210	220	230	240
	GTVFDSSKANGGPVTFPLSQVIPGWTEGVQLKEGGEATFYIPSNLAYREQAGDKIGPN					
g576-1.pep	250	260	270			
	ATLVFDVKLVKIGAPENAPAKQPDQVDIKKVN					
m576-1	250	260	270			
	ATLVFDVKLVKIGAPENAPAKQPAQVDIKKVN					

The following DNA sequence was identified in *N. meningitidis* <SEQ ID 30>:

a576-1.seq
 1 ATGAACACCA TTTTCAAAAT CAGCGCACTG ACCCTTTCCG CCGCTTTGGC
 51 ACTTTCCGCC TCGCGCAAAA AAGAAGCCGC CCCCGCATCT GCATCCGAAC
 101 CTGCCGCCGC TTCTTCCGCG CAGGGCGACA CCTCTTCGAT CGGCAGCACG
 151 ATGCAGCAGG CAAGCTATGC GATGGGCGTG GACATCGGAC GCTCCCTGAA
 201 GCAATGAAG GAACAGGGCG CGGAAATCGA TTTGAAAGTC TTTACCGAAG
 251 CCATGCAGGC AGTGTATGAC GGCAAAGAAA TCAAATGAC CGAAGAGCAG
 301 GCTCAGGAAG TCATGATGAA ATTCTTCAG GAACAACAGG CTAAAGCCGT
 351 AGAAAAACAC AAGGCGGACG CGAAGGCCAA TAAAGAAAAA GGCGAAGCCT
 401 TTCTGAAAGA AAATGCCGCC AAAGACGGCG TGAAGACCAC TGCTTCCGGC
 451 CTGCAATACA AAATCACCAA ACAGGGCGAA GGCAAACAGC CGACCAAGA
 501 CGACATCGTT ACCGTGGAAT ACGAAGGCCG CCTGATTGAC GGTACGGTAT
 551 TCGACAGCAG CAAAGCCAAC GCGGCGCCCG TCACCTTCCC TTGAGCCAA
 601 GTGATTCTGG GTTGACCGA AGGCGTACAG CTTCTGAAAG AAGGCGGCGA
 651 AGCCACGTTT TACATCCCGT CCAACCTTGC CTACCGCGAA CAGGCTGCGC
 701 GCGACAAAAT CGGCCCGAAC GCCACTTTGG TATTTGATGT GAACTGGTC

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751 AAAATCGGCG CACCCGAAAA CGCGCCCGCC AAGCAGCCGG CTCAGTCGA
801 CATCAAAAAA GTAAATTAA

This corresponds to the amino acid sequence <SEQ ID 31; ORF 576-1.a>:

a576-1.pep
1 MNTIFKISAL TLSAALALSA CGKKEAAPAS ASEPAASSA QGDTSSIGST
51 MQQASYAMGV DIGRSLKQMK EQGAEIDLKV FTEAMQAVYD GKEIKMTEEQ
101 AQEVMMKFLO EQQAKAVEKH KADAKANKEK GEAFLENAA KDGVKTTASG
151 LQYKITKQGE GKQPTKDDIV TVEYEGRLID GTVFDSSKAN GGPVTFPLSQ
201 VILGWTEGVQ LLKEGGEATF YIPSNLAYRE QGAGDKIGPN ATLVFDVKLV
251 KIGAPENAPA KQPAQVDIKK VN*

a576-1/m576-1 ORFs 576-1 and 576-1.a 99.6% identity in 272 aa overlap

a576-1.pep	10	20	30	40	50	60
	MNTIFKISALTLSAALALSACGKKEAAPASASEPAAASSAQGDTSSIGSTMQQASYAMGV					
m576-1	10	20	30	40	50	60
	MNTIFKISALTLSAALALSACGKKEAAPASASEPAAASSAQGDTSSIGSTMQQASYAMGV					
a576-1.pep	70	80	90	100	110	120
	DIGRSLKQMKEQGAEIDLKVTEAMQAVYDGKEIKMTEEQAQEVMMKFLOEQQAKAVEKH					
m576-1	70	80	90	100	110	120
	DIGRSLKQMKEQGAEIDLKVTEAMQAVYDGKEIKMTEEQAQEVMMKFLOEQQAKAVEKH					
a576-1.pep	130	140	150	160	170	180
	KADAKANKEKGEAFLENAAKDGVKTTASGLQYKITKQEGGKQPTKDDIVTVEYEGRLID					
m576-1	130	140	150	160	170	180
	KADAKANKEKGEAFLENAAKDGVKTTASGLQYKITKQEGGKQPTKDDIVTVEYEGRLID					
a576-1.pep	190	200	210	220	230	240
	GTVFDSSKANGGPVTFPLSQVILGWTEGVQLLKEGGEATFYIPSNLAYREQGAGDKIGPN					
m576-1	190	200	210	220	230	240
	GTVFDSSKANGGPVTFPLSQVILGWTEGVQLLKEGGEATFYIPSNLAYREQGAGDKIGPN					
a576-1.pep	250	260	270			
	ATLVFDVKLVKIGAPENAPAKQPAQVDIKKVN					
m576-1	250	260	270			
	ATLVFDVKLVKIGAPENAPAKQPAQVDIKKVN					

919 and 919-2

gnm43.seq

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 32>:

m919.seq
1 ATGAAAAAAT ACCTATTCCG CGCCGCCCTG TACGGCATCG CCGCCGCCAT
51 CCTCGCCGCC TGCCAAAGCA AGAGCATCCA AACCTTTCCG CAACCCGACA
101 CATCCGTCAT CAACGGCCCG GACCGGCCCG TCGGCATCCC CGACCCCGCC
151 GGAACGACGG TCGGCGGCCG CGGGGCCGTC TATACCGTTG TACCGCACCT
201 GTCCCTGCCC CACTGGGCGG CGCAGGATT CGCCAAAAGC CTGCAATCCT
251 TCCGCCTCGG CTGCGCCAAT TTGAAAAACC GCCAAGGCTG GCAGGATGTG
301 TGCGCCCAAG CCTTTCAAAC CCCCGTCCAT TCCTTTCAGG CAAAACAGTT
351 TTTTGAACGC TATTTACGCG CGTGGCAGGT TGCAGGCAAC GGAAGCCTTG

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401 CCGGTACGGT TACCGGCTAT TACGAACCGG TGCTGAAGGG CGACGACAGG
451 CGGACGGCAC AAGCCCCTT CCGATTAC GGTATTCCCG ACGATTTTAT
501 CTCCGTCCCC CTGCCTGCCG GTTTGCGGAG CGGAAAAGCC CTTGTCCGCA
551 TCAGGCAGAC GGGAAAAAAC AGCGGCACAA TCGACAATAC CGGCGGCACA
601 CATACCGCCG ACCTCTCCCG ATTCCCATC ACCGCGCGCA CAACAGCAAT
651 CAAAGGCAGG TTTGAAGGAA GCCGCTTCCT CCCCTACCAC ACGCGCAACC
701 AAATCAACGG CGGCGCGCTT GACGGCAAAG CCCCATACT CGGTTACGCC
751 GAAGACCCTG TCGAACTTT TTTATGCAC ATCCAAGGCT CGGGCCGTCT
801 GAAAACCCCG TCCGGCAAAT ACATCCGCAT CGGCTATGCC GACAAAAACG
851 AACATCCyTA CGTTTCCATC GGACGCTATA TGGCGGATAA GGGCTACCTC
901 AAACCTCGAC AAACCTCCAT GCAGGGCATT AAGTCTTATA TGGCGCAAAA
951 TCCGCAACGC CTCGCCGAAG TTTGGGTCA AAACCCAGC TATATCTTTT
1001 TCCGCGAGCT TGCCGGAAGC AGCAATGACG GCCCTGTCGG CGCACTGGGC
1051 ACGCCGCTGA TGGGGGAATA TGCCGGCGCA GTCGACCGGC ACTACATTAC
1101 CTTGGGTGCG CCCTTATTG TCGCCACCGC CCATCCGGTT ACCCGCAAAG
1151 CCCTCAACCG CCTGATTATG GCGCAGGATA CCGGCAGCGC GATTAAAGGC
1201 GCGGTGCGCG TGGATTATTT TTGGGGATAC GCGCAGGAAG CCGGCGAACT
1251 TGCCGGCAAA CAGAAAACCA CGGGATATGT CTGGCAGCTC CTACCCAACG
1301 GTATGAAGCC CGAATACCGC CCGTAA

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This corresponds to the amino acid sequence <SEQ ID 33; ORF 919>:

m919.pep

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1 MKKYLFRAL YGIAAILAA CQSKSIQTFP QPDTSVINGP DRPVGIPDPA
51 GTTVGGGAV YTVVPHLSLP HWAAQDFAKS LQSFRLGCAN LKNRQGWQDV
101 CAQAFQTPVH SFQAKQFFER YFTPWQVAGN GSLAGTVTGY YEPVLKGDDR
151 RTAQARFPIY GIPDDFISVP LPAGLRSKGA LVRIROTGKN SGTIDNTGGT
201 HTADLSRFPI TARTTAIKGR FEGRSFLPYH TRNQINGGAL DGKAPILGYA
251 EDPVELFFMH IQGSGRLKTP SGKYIRIGYA DKNEHPYVSI GRYMADKGYL
301 KLGQTSMQGI KSYMQRNPQR LAEVLGQNPS YIFFRELGS SNDGPVGALG
351 TPLMGEYAGA VDRHYITLGA PLFVATAHPV TRKALNRLIM AQDTGSAIKG
401 AVRVDYFWGY GDEAGELAGK QKTGYVWQL LPNGMKPEYR P*

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The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 34>:

m919-2.seq

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1 ATGAAAAAAT ACCTATTCCG CGCGCCCTCG TACGGCATCG CCGCCGCCAT
51 CCTCGCCGCC TGCCAAAGCA AGAGCATCCA AACCTTTCCG CAACCCGACA
101 CATCCGTCAT CAACGGCCCG GACCGGCCGG TCGGCATCCC CGACCCCGCC
151 GGAACGACGG TCGGCGGCGG CGGGGCCGTC TATACCGTTG TACCGCACCT
201 GTCCCTGCCC CACTGGGCGG CGCAGGATTT CGCCAAAGC CTGCAATCCT
251 TCCGCCTCGG CTGCGCCAAT TTGAAAAACC GCCAAGGCTG GCAGGATGTG
301 TCGGCCCAAG CCTTTCAAAC CCCCCTCCAT TCCTTTCAGG CAAAACAGTT
351 TTTTGAACGC TATTTACGC CGTGGCAGGT TGCAGGCAAC GGAAGCCTTG
401 CCGGTACGGT TACCGGCTAT TACGAACCGG TGCTGAAGGG CGACGACAGG
451 CGGACGGCAC AAGCCCCTT CCGATTAC GGTATTCCCG ACGATTTTAT
501 CTCCGTCCCC CTGCCTGCCG GTTTGCGGAG CGGAAAAGCC CTTGTCCGCA
551 TCAGGCAGAC GGGAAAAAAC AGCGGCACAA TCGACAATAC CGGCGGCACA
601 CATACCGCCG ACCTCTCCCG ATTCCCATC ACCGCGCGCA CAACAGCAAT
651 CAAAGGCAGG TTTGAAGGAA GCCGCTTCCT CCCCTACCAC ACGCGCAACC
701 AAATCAACGG CGGCGCGCTT GACGGCAAAG CCCCATACT CGGTTACGCC
751 GAAGACCCTG TCGAACTTT TTTATGCAC ATCCAAGGCT CGGGCCGTCT
801 GAAAACCCCG TCCGGCAAAT ACATCCGCAT CGGCTATGCC GACAAAAACG
851 AACATCCCTA CGTTTCCATC GGACGCTATA TGGCGGATAA GGGCTACCTC
901 AAACCTCGAC AAACCTCCAT GCAGGGCATT AAGTCTTATA TGGCGCAAAA
951 TCCGCAACGC CTCGCCGAAG TTTGGGTCA AAACCCAGC TATATCTTTT
1001 TCCGCGAGCT TGCCGGAAGC AGCAATGACG GCCCTGTCGG CGCACTGGGC
1051 ACGCCGCTGA TGGGGGAATA TGCCGGCGCA GTCGACCGGC ACTACATTAC
1101 CTTGGGTGCG CCCTTATTG TCGCCACCGC CCATCCGGTT ACCCGCAAAG
1151 CCCTCAACCG CCTGATTATG GCGCAGGATA CCGGCAGCGC GATTAAAGGC

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1201 GCGGTGCGCG TGGATTATTT TTGGGGATAC GGCGACGAAG CCGGCGAACT
 1251 TGCCGGCAAA CAGAAAACCA CGGGATATGT CTGGCAGCTC CTACCCAACG
 1301 GTATGAAGCC CGAATACCGC CCGTAA

This corresponds to the amino acid sequence <SEQ ID 35; ORF 919-2>:

m919-2.pep

1 MKKYLFRAL YGIAAAILAA CQSKSIQTFP QPDTSVINGP DRPVGIPDPA
 51 GTTVGGGAV YTVVPHLSLP HWAAQDFAKS LQSFRLGCAN LKNRQGWQDV
 101 CAQAFQTPVH SFQAKQFFER YFTPWQVAGN GSLAGTVTGY YEPVLKGDGR
 151 RTAQARFPIY GIPDDFISVP LPAGLRSGKA LVRIRQTGKN SGTIDNTGGT
 201 HTADLSRFPI TARTTAIKGR FEGRSFLPYH TRNQINGGAL DGKAPILGYA
 251 EDPVELFFMH IQGSGRLKTP SGKYIRIGYA DKNEHPYVSI GRYMADKGYL
 301 KLGQTSMQGI KSYMQRNPQR LAEVLGQNPS YIFFRELAGS SNGPVGALG
 351 TPLMGEYAGA VDRHYITLGA PLFVATAHPV TRKALNRLIM AQDTGSAIKG
 401 AVRVDYFWGY GDEAGELAGK QKTTGYVWQL LPNGMKPEYR P*

The following partial DNA sequence was identified in *N.gonorrhoeae* <SEQ ID 36>:

g919.seq

1 ATGAAAAAAC ACCTGCTCCG CTCGCCCTG TACGGcatCG CCGCCgccAT
 51 CctcgCCGCC TGCCAAAgca gGAGCATCCA AACCTTTCCG CAACCCGACA
 101 CATCCGTCAT CAACGGCCCG GACCGGCCCG CCGGCATCCC CGACCCCGCC
 151 GGAACGACGG TTGCCGCGCG CGGGGCCGTC TATACCGTTG TGCCGCACTT
 201 GTCCATGCCC CACTGGGCGG CGCaggATTT TGCCAAAGC CTGCAATCCT
 251 TCCGCCTCGG CTGCGCCAAT TTGAAAAACC GCCAAGGCTG GCAGGATGTG
 301 TGCGCCCAAG CCTTTCAAAC CCCCGTGCAT TCCTTTCAGG CAAAGcGgTT
 351 TTTTGAACGC TATTTCACGC cgtGGCaggt tgcaggcaAC GGAAGcCTTG
 401 Caggtacggt TACCGGCTAT TACGAACCGG TGCTGAAGGG CGACGGCAGG
 451 CGGACGGAAC GGGCCCCGCTT CCCGATTAC GGTATTCCCG ACGATTITAT
 501 TCCTCGTCCG CTGCCTGCCG GTTTGCGGGG CGGAAAAAAC CTGTGTCGCA
 551 CTAGGCAGac ggGGA AAAAC AGCGGCACGA TCGACAATGC CGGCGGCACG
 601 CATACGCGCG ACCTCTCCCG ATTCCCCATC ACCGCGCGCA CAACGGcaat
 651 caaaGGCAGG TTTGAaggAA GCCGCTTCTT CCTTACCAC ACGCGCAACC
 701 AAAtcaacGG CGGCgcgCTT GACGGCAAag cccCCATCCT CggttacgcC
 751 GAagaccCcg tcgaacttTT TTTCATGCAC AtccaaggCT CGGGCCGCTT
 801 GAAAACCCcg tccggcaaat acatCCGCaT cggTaagcc gacAAAAACG
 851 AACAtccgTa tgtttccatc ggACGctaTA TGGCGGACAA AGGCTACCTC
 901 AAGctcgggc agACCTCGAT GCAGGgcac aaagcCTATA TGCGGCAAAA
 951 TCCGCAACGC CTCGCGAAG TTTTGGGTCA AAACCCAGC TATATCTTTT
 1001 TCCGCGAGCT TGCCGGAAGC GGCAATGAGG GCCCGTCCG CGCACTGGGC
 1051 ACGCCACTGA TGGGGGAATA CGCCGGCGCA ATCGACCGGC ACTACATTAC
 1101 CTTGGGCGCG CCCTTATTTG TCGCCACCGC CCATCCGGTT ACCCGCAAAG
 1151 CCCTCAACCG CCTGATTATG GCGCAGGATA CAGGCAGCGC GATCAAAGGC
 1201 GCGGTGCGCG TGGATTATTT TTGGGGTTAC GGCGACGAAG CCGGCGAACT
 1251 TGCCGGCAAA CAGAAAACCA CGGGATACGT CTGGCAGCTC CTGCCCAACG
 1301 GCATGAAGCC CGAATACCGC CCGTGA

This corresponds to the amino acid sequence <SEQ ID 37; ORF 919.ng>:

g919.pep

1 MKKHLLRSAL YGIAAAILAA CQSRSIQTFP QPDTSVINGP DRPAGIPDPA
 51 GTTVAGGGAV YTVVPHLSMP HWAAQDFAKS LQSFRLGCAN LKNRQGWQDV
 101 CAQAFQTPVH SFQAKRFFER YFTPWQVAGN GSLAGTVTGY YEPVLKGDGR
 151 RTERARFPIY GIPDDFISVP LPAGLRGGKN LVRIRQTGKN SGTIDNAGGT
 201 HTADLSRFPI TARTTAIKGR FEGRSFLPYH TRNQINGGAL DGKAPILGYA
 251 EDPVELFFMH IQGSGRLKTP SGKYIRIGYA DKNEHPYVSI GRYMADKGYL
 301 KLGQTSMQGI KAYMRNPQR LAEVLGQNPS YIFFRELAGS GNEGVPVAGL
 351 TPLMGEYAGA IDRHYITLGA PLFVATAHPV TRKALNRLIM AQDTGSAIKG
 401 AVRVDYFWGY GDEAGELAGK QKTTGYVWQL LPNGMKPEYR P*

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 38>:
a919.seq

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```

1  ATGAAAAAAT ACCTATTCCG CGCCGCCCTG TCGGCATCG CCGCCGCCAT
51  CCTCGCCGCC TGCCAAAGCA AGAGCATCCA AACCTTTCCG CAACCCGACA
101 CATCCGTCAT CAACGGCCCG GACCGGCCGG TCGGCATCCC CGACCCGCCC
151 GGAACGACGG TCGCGGCGGG CGGGGCCGTT TATACCGTTG TGCCGCACCT
201 GTCCCTGCCC CACTGGGCGG CGCAGGATT CGCCAAAAGC CTGCAATCCT
251 TCCGCCTCGG CTGCGCCAAT TTGAAAAACC GCCAAGGCTG GCAGGATGTG
301 TCGGCCCAAG CCTTTCAAAC CCCCGTCCAT TCCGTTGAGG CAAAACAGTT
351 TTTTGAACGC TATTTACGCG CGTGGCAGGT TGCAGGCAAC GGAAGCCTTG
401 CCGGTACGGT TACCGGCTAT TACGAGCCGG TGCTGAAGGG CGACGACAGG
451 CGGACGGCAC AAGCCCGCTT CCCGATTTAC GGTATTCGCG ACGATTTTAT
501 CTCGCTCCCC CTGCCTGCCG GTTTGCGGAG CGGAAAAGCC CTTGTCCGCA
551 TCAGGCAGAC GGGAAAAAAC AGCGGCACAA TCGACAATAC CGGCGGCACA
601 CATAACGCGG ACCTCTCCCA ATTCCCATC ACTGCGCGCA CAACGCCAAT
651 CAAAGGCAGG TTTGAAGGAA GCCGCTTCCT CCCCTACCAC ACGCGCAACC
701 AAATCAACGG CGGCGCGCTT GACGGCAAAG CCCCATACT CGGTACGCC
751 GAAGACCCG TCGAACTTTT TTTTATGCAC ATCCAAGGCT CGGGCCGTCT
801 GAAAACCCCG TCCGCAAAAT ACATCCGCAT CGGCTATGCC GACAAAAACG
851 AACATCCCTA CGTTTCCATC GGACGCTATA TGGCGGACAA AGGCTACCTC
901 AAGCTCGGGC AGACCTCGAT GCAGGGCATC AAAGCCTATA TGCAGCAAAA
951 CCCGCAACGC CTCGCCGAAG TTTTGGGGCA AAACCCAGC TATATCTTTT
1001 TCCGAGAGCT TACCGGAAGC AGCAATGACG GCCCTGTCGG CGCACTGGGC
1051 ACGCCGCTGA TGGGCGAGTA CGCCGGCGCA GTCGACCGGC ACTACATTAC
1101 CTTGGGCGGG CCCTATTGTC TCGCCACCGC CCATCCGGTT ACCCGCAAAG
1151 CCTCAACCG CCTGATTATG GCGCAGGATA CCGCAGCGC GATTAAAGGC
1201 GCGGTGCGCG TGGATTATTT TTGGGGATAC GGCGACGAAG CCGCGCAACT
1251 TGCCGGCAAA CAGAAAACCA CGGGATATGT CTGGCAGCTT CTGCCCAACG
1301 GTATGAAGCC CGAATACCGC CCGTAA

```

This corresponds to the amino acid sequence <SEQ ID 39; ORF 919.a>:

```

a919.pep
1  MKKYLFRAL CGIAAAILAA CQSKSIQTFP QPDTSVINGP DRPVGIPDPA
51  GTTVGGGGAV YTVVPHLSLP HWAAQDFAKS LQSFRLGCAN LKNRQGWQDV
101 CAQAFQTPVH SVQAKOFFER YFTPWQVAGN GSLAGTVTGY YEPVLKGDDR
151 RTAQARFPIY GIPDDFISVP LPAGLRSGKA LVRIRQTGKN SGTIDNTGGT
201 HTADLSQFPI TARTTAIKGR FEGRFLPYH TRNQINGGAL DGKAPILGYA
251 EDPVELFFMH IQSGRLKTP SGKYIRIGYA DKNEHPYVSI GRYMADKGYL
301 KLGQTSMQGI KAYMQQNPQR LAEVLGQNPS YIFRELTGS SNDGPVAGLG
351 TPLMGEYAGA VDRHYITLGA PLFVATAHPV TRKALNRLIM AQDTGSAIKG
401 AVRVDYFWGY GDEAGELAGK QKTTGYVWQL LPNGMKPEYR P*

```

m919/a919 ORFs 919 and 919.a showed a 98.6% identity in 441 aa overlap

```

m919.pep      10      20      30      40      50      60
MKKYLFRALYGIAAAILAACQSKSIQTFPQPDTSVINGPDRPVGIPDPAGTTVGGGGAV
|||||
a919          MKKYLFRALCGIAAAILAACQSKSIQTFPQPDTSVINGPDRPVGIPDPAGTTVGGGGAV
10      20      30      40      50      60

m919.pep      70      80      90      100     110     120
YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKOFFER
|||||
a919          YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSVQAKOFFER
70      80      90      100     110     120

m919.pep      130     140     150     160     170     180
YFTPWQVAGNGSLAGTVTGYEYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
|||||
a919          YFTPWQVAGNGSLAGTVTGYEYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
130     140     150     160     170     180

m919.pep      190     200     210     220     230     240
LVRIRQTGKNSGTIDNTGGTHTADLSRFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
|||||

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a919      LVRIRQTGKNSGTIDNTGGTHTADLSQFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
           190      200      210      220      230      240

           250      260      270      280      290      300
m919.pep  DGKAPILGYAEDPVELFFMHIIQSGRLKTPSGKYIRIGYADKNEHPYVSIGRYMADKGYL
           |||||||
a919      DGKAPILGYAEDPVELFFMHIIQSGRLKTPSGKYIRIGYADKNEHPYVSIGRYMADKGYL
           250      260      270      280      290      300

           310      320      330      340      350      360
m919.pep  KLGQTSMQGIKSYMRQNPQRLAEVLGQNPSYIFFRELAGSSNDGPVGGALGTPLMGEYAGA
           |||||||
a919      KLGQTSMQGIKAYMQNPQRLAEVLGQNPSYIFFRELTGSSNDGPVGGALGTPLMGEYAGA
           310      320      330      340      350      360

           370      380      390      400      410      420
m919.pep  VDRHYITLGAPLFVATAHPVTRKALNRLIMAQDTGSAIKGAVRVDFWGYGDEAGELAGK
           |||||||
a919      VDRHYITLGAPLFVATAHPVTRKALNRLIMAQDTGSAIKGAVRVDFWGYGDEAGELAGK
           370      380      390      400      410      420

           430      440
m919.pep  QKTTGYVWQLLPNGMKPEYRPX
           |||||||
a919      QKTTGYVWQLLPNGMKPEYRPX
           430      440

```

121 and 121-1

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 40>:

m121.seq

```

1  ATGGAACAC AGCTTTACAT CGGCATCATG TCGGGAACCA GCATGGACGG
51  GCGGATGCC GTACTGATAC GGATGGACGG CGGCAATGG CTGGGCGCGG
101 AAGGGCAGC CTTTACCCCC TACCCGCGCA GGTACGCCG CCAATTGCTG
151 GATTTGCAGG ACACAGGCGC AGACGAACTG CACCGCAGCA GGATTTGTGC
201 GCAAGAACTC AGCCGCCTAT ATGCGCAAAC CGCCGCCGAA CTGCTGTGCA
251 GTCAAAACCT CGCACCGTCC GACATTACCG CCTCGGCTG CCACGGGCAA
301 ACCGTCCGAC ACGCGCCGGA ACACGGTTAC AGCATACAGC TTGCCGATTT
351 GCCGCTGCTG GCGxxxxxxx xxxxxxxxxx xxxxxxxxxx xxxxxxxxxx
401 xxxxxxxxxx xxxxxxxxxx xxxxxxxxxx xxxxxxxxxx xxxxxxxxxx
451 xxxxxxxxxx xxxxxxxxxx xxxxxxxxxx xxxxxxxxxx xxxxxxxxxx
501 xxxxxxxxxx xxxxxxxxxx xxxxxxxxxx xxxxxxxxxx xxxxxxxxxx
551 xxxxxxxxxx xxxxxxxxxx xxxxxxxxxx xxxxxxxxxx xxxxxxxxxx
601 xxxxxxGAGC TTCCTTACGA CAAAACGGT GCAAAGTCGG CACAAGGCAA
651 CATATTGCCG CAACTGCTCG ACAGGCTGCT CGCCACCCG TATTTGCGAC
701 AACGCCACCC TAAAGCAGC GGGCGCGAAC TGTTGCCAT AAATTGGCTC
751 GAAACCTACC TTGACGCGCG CGAAAACCGA TACGACGTAT TGCGGACGCT
801 TTCCCGTTTT ACCGCGCAAA CCGTTTGCGA CGCCGTCTCA CACGAGCGG
851 CAGATGCCCG TCAAATGTAC ATTTGCGACG GCGGCATCCG CAATCCTGTT
901 TTAATGGCGG ATTTGGCAGA ATGTTTCGGC ACACGCGTTT CCCTGCACAG
951 CACCGCCGAC CTGAACCTCG ATCCGCAATG GGTGGAAGCC GCCGnATTTG
1001 CGTGTTGGC GCGGTGTTGG ATTAATCGCA TTCCCGGTAG TCCGCACAAA
1051 GCAACCGCGC CATCCAAACC GTGTATTCTG AnCGCGGGAT ATTATTATTG
1101 A

```

This corresponds to the amino acid sequence <SEQ ID 41; ORF 121>:

m121.pep

```

1  METQLYIGIM SGTSMGADA VLIRMDGGKW LGAEGHAFTP YPGRLLRQLL
51  DLQDTGADEL HRSRIISQEL SRLYAQTAAE LLCQNLAAPS DITALGCHGQ

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101 TVRHAPHEGY SIQLADLPLL Axxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx
151 xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx
201 xxQLPYDKNG AKSAQGNILP QLLDRLLAHP YFAQRHPKST GRELFAINWL
251 ETYLDGGENR YDVLRTLSRF TAQTVCDAYS HAAADARQMY ICDGGIRNPV
301 LMADLAECFG TRVSLHSTAD LNLDPQWVEA AXFAWLAACW INRIPGSPHK
351 ATGASKPCIL XAGYYY*

```

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 42>:

g121.seq

```

1 ATGGAAACAC AGCTTTACAT CGGCATTATG TCGGGAACCA GTATGGACGG
51 GGCGGATGCC GTGCTGGTAC GGATGGACGG CGGCAATGG CTGGGCGCGG
101 AAGGGCACGC CTTTACCCCC TACCTGACC GGTTCGCGCG CAAATTGCTG
151 GATTTCAGG ACACAGGCAC AGACGAATG CACCGCAGCA GGATGTTGTC
201 GCAAGAACTC AGCCGCCTGT ACGCGCAAAC CGCCGCCGAA CTGCTGTGCA
251 GTCAAAACCT CGCTCCGTGC GACATTACCG CCCTCGGCTG CCACGGGCAA
301 ACCGTCCGAC ACGCGCCGGA ACACGGTtac AGCATACAGC TTGCCGATTT
351 GCCGCTGCTG GCGGAACTGa cgcggatttt TACCGTCggc gacttcCGCA
401 GCCGCGACCT TGCTCCCGGC GGacaAGGTG CGCCGCTCGT CCCCgcCTTT
451 CACGAAGCCC TGTTCCGCGA TGACAGGGAA ACACGCGTGG TACTGAACAT
501 CGGCGGGATT GCCAACATCA GCGTACTCCC CCCCggCGCA CCCCgcTTCC
551 GCTTCGACAC AGGGCCGGGC AATATGCTGA TGGAcgcgtg gacgcaggca
601 cacTGGcagc TGCTTACGA CAAAacggt gcAAAGgcgg cacAAGGCAA
651 catattGCcg cAACTGCTCG gcaggctGCT CGCCcaccCG TATTTCAC
701 AACCCcacc aaAAGCACG GGgcGCGaac TgtttgcccT AAattggctc
751 gaaacctAcc ttgacggcgg cgaaaaccga tacgacgtat tgcggacgct
801 ttccccgatt accgcgcaaA ccgTttggga cgccgtctca CACGCAGCGG
851 CAGATGCCCG TCAATGTAC ATTTGCGGCG GCGGCATCCG CAATCCTGTT
901 TTAATGGCGG ATTTGGCAGA ATGTTTCGGC ACACGCGTTT CCCTGCACAG
951 CACCGCCGAA CTGAACCTCG ATCCTCAATG GGTGGAGGCG gccgCATttg
1001 cgtggttgG GCGGTGTTGG ATTAACCGCA TTCCCGGTAG TCCGCACAAA
1051 GCGACCGGCG CATCCAAACC GTGTATTCTG GCGCGGGGAT ATTATTATTG
1101 A

```

This corresponds to the amino acid sequence <SEQ ID 43; ORF 121.ng>:

g121.pep

```

1 METQLYIGIM SGTSMGDADA VLVRMDGGKW LGAEGHAFTP YPDLRRKLL
51 DLQDTGTDEL HRSRMLSQEL SRLYAQTAAE LLCSONLAPC DITALGCHGQ
101 TVRHAPHEGY SIQLADLPLL AELTRIFTVG DFRSRDLAAG GQGAPLVPAP
151 HEALFRDDRE TRVVLNIGGI ANISVLPPGA PAFGFDTPG NMLMDAWTQA
201 HWQLPYDKNG AKAAQGNILP QLLGRLLAHP YFSQPHPKST GRELFALNWL
251 ETYLDGGENR YDVLRTLSRF TAQTVWDAYS HAAADARQMY ICGGGIRNPV
301 LMADLAECFG TRVSLHSTAE LNLDPQWVEA AAFWLAACW INRIPGSPHK
351 ATGASKPCIL GAGYYY*

```

ORF 121 shows 73.5% identity over a 366 aa overlap with a predicted ORF (ORF121.ng) from *N. gonorrhoeae*:

m121/g121

	10	20	30	40	50	60
m121.pep	METQLYIGIMSGTSMGDADAVLIRMDGGKWLGAEHGAFTYPFGRLLRQQLDLQDTGADEL					
g121	METQLYIGIMSGTSMGDADAVLVRMDGGKWLGAEHGAFTYPFGRLLRQQLDLQDTGTDEL					
	10	20	30	40	50	60
	70	80	90	100	110	120
m121.pep	HRSRILSQELSRLYAQTAAELLCSONLAPSDITALGCHGQTVRHAPHEGYISQLADLPLL					
g121	HRSRILSQELSRLYAQTAAELLCSONLAPCDITALGCHGQTVRHAPHEGYISQLADLPLL					
	70	80	90	100	110	120
	130	140	150	160	170	180

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m121.pep  AXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
           | : : : : : : : : : : : : : : : : : : : : : :
g121       AELTRIFTVGDFRSRDLAAGGQGAPLVPAFHEALFRDDRETRVVLNIGGIANISVLPFGA
           130      140      150      160      170      180
           190      200      210      220      230      240
m121.pep  XXXXXXXXXXXXXXXXXXXXXXXLPYDKNGAKSAQGNILPQLLDRLLAHPYFAQRHPKST
           : : : : : : : : : : : : : : : : : : : : : :
g121       PAFGFDTGPGNMLMDAWTQAHWQLPYDKNGAKAAQGNILPQLLGRLLAHPYFSQPHPKST
           190      200      210      220      230      240
           250      260      270      280      290      300
m121.pep  GRELFAINWLETYLDGGENRYDVLRTLRSFTAQTVCDVSHAAADARQMYICDGGIRNPV
           | | | | : | | | | | | | | | | | | | | | | | | | | |
g121       GRELFALNWLETYLDGGENRYDVLRTLRSFTAQTVWDVSHAAADARQMYICGGGIRNPV
           250      260      270      280      290      300
           310      320      330      340      350      360
m121.pep  LMADLAECFGTRVSLHSTADLNLDPOWVEAAXFAWLAACWINRIPGSPHKATGASKPCIL
           | | | | : | | | | | | | | | | | | | | | | | | | | |
g121       LMADLAECFGTRVSLHSTAE LNLDPOWVEAAAFWLAACWINRIPGSPHKATGASKPCIL
           310      320      330      340      350      360

m121.pep  XAGYYYYX
           | | | | |
g121       GAGYYYYX

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 44>:

```

a121.seq
1  ATGGAACAC AGCTTTACAT CGGCATCATG TCGGGAACCA GCATGGACGG
51  GGCGGATGCC GTACTGATAC GGATGGACGG CGGCAAATGG CTGGGCGCGG
101 AAGGGCACGC CTTTACCCCC TACCCGCGCA GGTACGCGG CAAATTGCTG
151 GATTTCAGAG ACACAGGCGC GGACGAACTG CACCGCAGCA GGATGTTGTC
201 GCAAGAACTC AGCCGCTGTG ACGCGCAAAC CGCCGCGGAA CTGCTGTGCA
251 GTCAAAACCT CGCGCCGTCC GACATTACCG CCCTCGGCTG CCACGGGCAA
301 ACCGTCAGAC ACGCGCCGGA ACACAGTTAC AGCGTACAGC TTGCCGATT
351 GCCGCTGCTG GCGGAACGGA CTCAGATTTT TACCGTCGGC GACTTCCGCA
401 GCCGCGACCT TGCGGCCGGC GGACAAGGCG CGCCGCTCGT CCCGCTTTT
451 CACGAAGCCC TGTTCCGCGA CGACAGGGAA ACACGCGCGG TACTGAACAT
501 CGGCGGGATT GCCAACATCA GCGTACTCCC CCCCAGCGCA CCCGCCTTCG
551 GCTTCGACAC AGGACCGGGC AATATGCTGA TGGACGCGTG GATGCAGGCA
601 CACTGGCAGC TTCCTTACGA CAAAACGGT GCAAAGGCGG CACAAGGCAA
651 CATATTGCCG CAACTGCTCG ACAGGCTGCT CGCCACCCG TATTTCGCAC
701 AACCCACCC TAAAGCACG GGGCGGAAC TGTTTGCCCT AAATTGGCTC
751 GAAACCTACC TTGACGGCGG CGAAAACCGA TACGACGTAT TCGGACGCT
801 TTCCCGATTG ACCGCGCAA CCGTTTTCGA CGCCGTCTCA CACGACGCG
851 CAGATGCCCG TCAATGTAC ATTTGCGGCG GCGGCATCCG CAATCCTGTT
901 TTAATGGCGG ATTTGGCAGA ATGTTTCGGC ACACGCGTTT CCTGACACG
951 CACCGCGGAA CTGAACCTCG ATCCGCAATG GGTAGAAGCC GCCGCGTTG
1001 CATGGATGGC GCGGTGTTGG GTCAACCGCA TTCCCGGTAG TCCGCACAAA
1051 GCAACCGCGG CATCCAAACC GTGTATTCTG GCGCGGGAT ATTATTATTG
1101 A

```

This corresponds to the amino acid sequence <SEQ ID 45; ORF 121.a>:

```

a121.pep
1  METQLYIGIM SGTSMGADA VLIRMDGGKW LGAEGHAFTP YPGRLRRLKLL
51  DLQDTGADEL HRSRMLSQEL SRLYAQTAAE LLCSONLAPS DITALGCHGQ
101 TVRHAPEHSY SVQLADLPLL AERTQIFTVG DFRSRDLAAG GQGAPLVPAF
151 HEALFRDDRE TRAVLNIGGI ANISVLPPDA PAFGFDTGPG NMLMDAWMQA
201 HWQLPYDKNG AKAAQGNILP QLLDRLLAHP YFAQPHPKST GRELFALNWL
251 ETYLDGGENR YDVLRTLRSF TAQTVFDAVS HAAADARQMY ICGGGIRNPV
301 LMADLAECFG TRVSLHSTAE LNLDPOWVEA AFAWMAACW VNRIIPGSPHK

```


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351 ATGASKPCIL GAGYYY*

m121/a121 ORFs 121 and 121.a 74.0% identity in 366 aa overlap

m121.pep	10	20	30	40	50	60
	METQLYIGIMSGTSMDGADAVLIRMDGGKWLGAEGHAFTYPYGRRLRQLLDLQDTGADEL					
a121	10	20	30	40	50	60
	METQLYIGIMSGTSMDGADAVLIRMDGGKWLGAEGHAFTYPYGRRLRQLLDLQDTGADEL					
m121.pep	70	80	90	100	110	120
	HRSRILSQELSRLYAQTAELLCSQNLAPSDITALGCHGQTVRHAPEHGYSIQIADLPL					
a121	70	80	90	100	110	120
	HRSRMLSQELSRLYAQTAELLCSQNLAPSDITALGCHGQTVRHAPEHSYSVQLADLPL					
m121.pep	130	140	150	160	170	180
	AXXX					
a121	130	140	150	160	170	180
	AERTQIFTVGDFRSRDLAAGGQGAFLVPAFHEALFRDDRETRAVLNIGGIANISVLPPDA					
m121.pep	190	200	210	220	230	240
	XXXXXXXXXXXXXXXXXXXXXXXXXQLPYDKNGAKSAQGNILPQLLDRLLAHPYFAQRHPKST					
a121	190	200	210	220	230	240
	PAFGFDTGPGNMLMDAWMQAHWQLPYDKNGAKAAQGNILPQLLDRLLAHPYFAQPHPKST					
m121.pep	250	260	270	280	290	300
	GRELFAINWLETYLDGGENRYDVLRLTSLRFTAQTVCDASHAAADARQMYICDGGIRNPV					
a121	250	260	270	280	290	300
	GRELFALNWLETYLDGGENRYDVLRLTSLRFTAQTVFDAVSHAAADARQMYICGGGIRNPV					
m121.pep	310	320	330	340	350	360
	LMADLAECFGTRVSLHSTADNLDPQWVEAAXFAWLAACWINRIPGSPHKATGASKPCIL					
a121	310	320	330	340	350	360
	LMADLAECFGTRVSLHSTAEINLDPQWVEAAAFAWMAACWVNRIIPGSPHKATGASKPCIL					
m121.pep	XAGYYYX					
a121	GAGYYYX					

Further work revealed the DNA sequence identified in *N. meningitidis* <SEQ ID 46>:

m121-1.seq

1	ATGGAACAC	AGCTTTACAT	CGGCATCATG	TCGGGAACCA	GCATGGACGG
51	GGCGGATGCC	GTACTGATAC	GGATGGACGG	CGGCAATGG	CTGGGCGCGG
101	AAGGGCAGCG	CTTTACCCCG	TACCCCGGCA	GGTTACGCCG	CCAATTGCTG
151	GATTTGCAGG	ACACAGGCGC	AGACGAAGTG	CACCGCAGCA	GGATTTGTCT
201	GCAAGAACTC	AGCCGCTTAT	ATGCGCAAAAC	CGCCGCGGAA	CTGCTGTGCA
251	GTCAAAACCT	CGCACCGTCC	GACATTACCG	CCCTCGGCTG	CCACGGGCAA
301	ACCGTCCGAC	ACGCGCCGGA	ACACGGTTAC	AGCATACAGC	TTGCCGATTT
351	GCCGCTGCTG	GCGGAACGGA	CGCGGATTTT	TACCGTCGCG	GACTTCCGCA
401	GCCGCGACCT	TGCGGCCGGC	GGACAAGGCG	CGCCACTCGT	CCCCGCCTTT
451	CACGAAGCCC	TGTTCCGCGA	CAACAGGGAA	ACACGCGCGG	TACTGAACAT
501	CGGCGGGATT	GCCAACATCA	GCGTACTCCC	CCCCGACGCA	CCCGCCTTCG
551	GCTTCCGACAC	AGGCGCGGGC	AATATGCTGA	TGGACGCGTG	GACGCAAGGCA
601	CACTGGCAGC	TTCTTACGA	CAAAAACGGT	GCAAAGGCGG	CACAAGGCAA
651	CATATTGCCG	CAACTGCTCG	ACAGGCTGCT	CGCCACCCG	TATTTCGCAC
701	AACCCACCC	TAAAAGCACG	GGGCGCGAAC	TGTTTGCCCT	AAATTGGCTC
751	GAAACCTACC	TTGACGGCGG	CGAAAACCGA	TACGACGTAT	TGCGGACGCT

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```

801 TTCCCGTTTT ACCGCGCAAA CCGTTTGC GA CGCCGTCTCA CACGCAGCGG
851 CAGATGCCCC TCAAATGTAC ATTTGCGGCG GCGGCATCCG CAATCCTGTT
901 TTAATGGCGG ATTTGGCAGA ATGTTTCGGC ACACGCGTTT CCCTGCACAG
951 CACCGCCGAC CTGAACCTCG ATCCGCAATG GGTGGAAGCC GCCGNATTG
1001 CGTGGTTGGC GGC GTTGG ATTAATCGCA TTCCCGGTAG TCCGCACAAA
1051 GCAACCGGCG CATCCAAACC GTGTATTCTG ANCGCGGGAT ATTATTATTG
1101 A

```

This corresponds to the amino acid sequence <SEQ ID 47; ORF 121-1>:

```

m121-1.pep
1 METQLYIGIM SGTSMGDADA VLIRMDGGKW LGAEGHAFTP YPGRLLRQLL
51 DLQDTGADEL HRSRILSQEL SRLYAQTAAE LLCQNLAAPS DITALGCHGQ
101 TVRHAPHEGY SIQLADLPLL AERTRIFTVG DFRSRDLAAG GQGAPLVPAF
151 HEALFRDNRE TRAVLNIGGI ANISVLPPDA PAFGFDTPG NMLMDAWTQA
201 HWQLPYDKNG AKAAQGNILP QLLDRLLAHP YFAQPHPKST GRELFALNWL
251 ETYLDGGENR YDVLRTLSRF TAQTVCDAVS HAAADARQMY ICGGGIRNPV
301 LMADLAECFG TRVSLHSTAD LNLDPQWVEA AXFAWLAACW INRIPGSPHK
351 ATGASKPCIL XAGYYY*

```

m121-1/g121 ORFs 121-1 and 121-1.ng showed a 95.6% identity in 366 aa overlap

```

          10      20      30      40      50      60
m121-1.pep METQLYIGIMSGTSMGDADAVLIRMDGGKWLGAEGHAFTYPGRLLRQLLDLQDTGADEL
          |||
g121 METQLYIGIMSGTSMGDADAVLRMDGGKWLGAEGHAFTYPDRLRRKLLDLQDTGTDEL
          10      20      30      40      50      60

          70      80      90      100     110     120
m121-1.pep HRSRILSQELSRLYAQTAAELLCQNLAAPSDITALGCHGQTVRHAPHEGYSIQLADLPLL
          |||
g121 HRSRILSQELSRLYAQTAAELLCQNLAAPCDITALGCHGQTVRHAPHEGYSIQLADLPLL
          70      80      90      100     110     120

          130     140     150     160     170     180
m121-1.pep AERTRIFTVGDFRSRDLAAGGQGAPLVPAFHEALFRDNRETRAVLNIGGIANISVLPPDA
          ||
g121 AELTRIFTVGDFRSRDLAAGGQGAPLVPAFHEALFRDDRETRVVLNIGGIANISVLPPGA
          130     140     150     160     170     180

          190     200     210     220     230     240
m121-1.pep PAFGFDTPGPNMLMDAWTQAHWQLPYDKNGAKAAQGNILPQLLDRLLAHPYFAQPHPKST
          |||
g121 PAFGFDTPGPNMLMDAWTQAHWQLPYDKNGAKAAQGNILPQLLGRLLAHPYFSQPHPKST
          190     200     210     220     230     240

          250     260     270     280     290     300
m121-1.pep GRELFALNWLETYLDGGENRYDVLRTLSRFTAQTVCDAVSHAAADARQMYICGGGIRNPV
          |||
g121 GRELFALNWLETYLDGGENRYDVLRTLSRFTAQTVWDVAVSHAAADARQMYICGGGIRNPV
          250     260     270     280     290     300

          310     320     330     340     350     360
m121-1.pep LMADLAECFGTRVSLHSTADLNLDPQWVEAAXFAWLAACWINRIPGSPHKATGASKPCIL
          |||
g121 LMADLAECFGTRVSLHSTAEINLDPQWVEAAAFWLAACWINRIPGSPHKATGASKPCIL
          310     320     330     340     350     360

m121-1.pep XAGYYYYX
          |||||
g121 GAGYYYYX

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 48>:

```
a121-1.seq
1  ATGGAAACAC AGCTTTACAT CGGCATCATG TCGGGAACCA GCATGGACGG
51  GGGCGATGCC GTACTGATAC GGATGGACGG CGGCAAATGG CTGGGCGCGG
101 AAGGGCACGC CTTTACCCCC TACCCCGGCA GGTTACGCCG CAAATGTGCTG
151 GATTTCAGG ACACAGGCGC GGACGAACTG CACCGCAGCA GGATGTTGTC
201 GCAAGAACTC AGCCGCCTGT ACGCGCAAAC CGCCGCCGAA CTGCTGTGCA
251 GTCAAAACCT CGCGCCGTCC GACATTACCG CCCTCGGCTG CCACGGGCAA
301 ACCGTCAGAC ACGCGCCGGA ACACAGTTAC AGCGTACAGC TTGCCGATTT
351 GCCGCTGCTG GCGGAACGGA CTCAGATTTT TACCGTCGGC GACTTCCGCA
401 GCCGCGACCT TCGCGCCGGC GGACAAGGCG CGCCGCTCGT CCCCCTTTT
451 CACGAAGCCC TGTTCGCGCA CGACAGGGAA ACACGCGCGG TACTGAACAT
501 CGGCGGGATT GCCAACATCA GCGTACTCCC CCCCAGCGCA CCCGCTTCG
551 GCTTCGACAC AGGACCGGGC AATATGCTGA TGGACGCGTG GATGCAGGCA
601 CACTGGCAGC TTCCTTACGA CAAAACGGT GCAAAGGCGG CACAAGGCAA
651 CATATTGCCG CAACTGCTCG ACAGGCTGCT CGCCCACCCG TATTTCGCAC
701 AACCCACCC TAAAAGCACG GGGCGCGAAC TGTTTGCCCT AAATTGGCTC
751 GAAACCTACC TTGACGGCGG CGAAAACCGA TACGACGTAT TCGGACGCT
801 TTCCCGATTC ACCGCGCAAA CCGTTTCGA CGCCGCTCA CACGACGCG
851 CAGATGCCG TCAAAATGAC ATTTGCGGCG GCGGCATCCG CAATCCTGTT
901 TTAATGGCGG ATTTGGCAGA ATGTTTCGGC ACACGCGTTT CCTGACAG
951 CACCGCCGAA CTGAACCTCG ATCCGCAATG GGTAGAGCC GCCGCGTTCG
1001 CATGGATGGC GCGGTGTTGG GTCAACCGCA TTCCCGGTAG TCCGCACAAA
1051 GCAACCGGCG CATCAAACC GTGATTCTG GCGCGGGAT ATTATTATTG
1101 A
```

This corresponds to the amino acid sequence <SEQ ID 49; ORF 121-1.a>:

```
a121-1.pep
1  METQLYIGIM SGTSMDGADA VLIRMDGGKW LGAEGHAFTP YPGRLLRRKLL
51  DLQDTGADEL HRSRMLSQEL SRLYAQTAAE LLCSONLAPS DITALGCHGQ
101 TVRHAPEHSY SVQLADLPLL AERTQIFTVG DFRSRDLAAG GQGAPLVPF
151 HEALFRDDRE TRAVLNIGGI ANISVLPPDA PAFGFDTPG NMLMDAWMQA
201 HWQLPYDKNG AKAAQGNILP QLLDRLLAHP YFAQPHPKST GRELFALNWL
251 ETYLDGGENR YDVLRTLSRF TAQTVFDAVS HAAADARQMY ICGGGIRNPV
301 LMADLAECFG TRVSLHSTAE LNLDPQWVEA AFAFWMAACW VNRIPGSPHK
351 ATGASKPCIL GAGYYY*
```

m121-1/a121-1 ORFs 121-1 and 121-1.a showed a 96.4% identity in 366 aa overlap

	10	20	30	40	50	60
m121-1.pep	METQLYIGIMSGTSMDGADAVLIRMDGGKWLGAEHGAFTPYPGRLRRQLLDLQDTGADEL					
a121-1	METQLYIGIMSGTSMDGADAVLIRMDGGKWLGAEHGAFTPYPGRLRRQLLDLQDTGADEL					
	10	20	30	40	50	60
	70	80	90	100	110	120
m121-1.pep	HRSRILSQELSRLYAQTAAELLCSONLAPSDITALGCHGQTVRHAPEHGYISQLADLPLL					
a121-1	HRSRILSQELSRLYAQTAAELLCSONLAPSDITALGCHGQTVRHAPEHSYSVQLADLPLL					
	70	80	90	100	110	120
	130	140	150	160	170	180
m121-1.pep	AERTRIFTVGDFRSRDLAAGGQAPLVPFHEALFRDNRETRAVLNIGGIANISVLPPDA					
a121-1	AERTQIFTVGDFRSRDLAAGGQAPLVPFHEALFRDDRETRAVLNIGGIANISVLPPDA					
	130	140	150	160	170	180
	190	200	210	220	230	240
m121-1.pep	PAFGFDTPGNMLMDAWTQAHWQLPYDKNGAKAAQGNILPQLLDRLLAHPYFAQPHPKST					
a121-1	PAFGFDTPGNMLMDAWMQAHWQLPYDKNGAKAAQGNILPQLLDRLLAHPYFAQPHPKST					
	190	200	210	220	230	240

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	250	260	270	280	290	300
m121-1.pep	GREL	FALN	WLET	YLDG	GENR	YDVL
a121-1	GREL	FALN	WLET	YLDG	GENR	YDVL
	250	260	270	280	290	300
	310	320	330	340	350	360
m121-1.pep	LMAD	LAEC	FGTR	VS	LHST	ADLN
a121	LMAD	LAEC	FGTR	VS	LHST	AE
	310	320	330	340	350	360
m121-1.pep	XAG	YYY	YX			
a121	GAG	YYY	YX			

128 and 128-1

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 50>:

m128.seq (partial)

```

1  ATGACTGACA  ACGCACTGCT  CCATTGGGC  GAAGAACCCC  GTTTTGATCA
51  AATCAAAACC  GAAGACATCA  AACCCGCCCT  GCAAACCGCC  ATCGCCGAAG
101 CGCGCGAACA  AATCGCCGCC  ATCAAAGCCC  AAACGCACAC  CGGCTGGGCA
151 AACACTGTCT  AACCCCTGAC  CGGCATCACC  GAACGCGTCG  GCAGGATTTG
201 GGGCGTGGTG  TCGCACCTCA  ACTGCGTCGC  CGACACGCCC  GAACTGCGCG
251 CCGTCTATAA  CGAACTGATG  CCCGAAATCA  CCGTCTTCTT  CACCGAAATC
301 GGACAAGACA  TCGAGCTGTA  CAACCGCTTC  AAAACCATCA  AAAATTCCCC
351 CGAATTCGAC  ACCCTCTCCC  CCGCACAAAA  AACCAACTC   AACCAC
1   TACGCCAGCG  AAAAACTGCG  CGAAGCCAAA  TACGCGTTCA  GCGAAACCGA
51  wGTCAAAAAA  TAYTTCCCYG  TCGGCAAwGT  ATTAACGGA   CTGTTCCGCC
101 AAmTCAAAAA  ACTmTACGGC  ATCGGATTTA  CCGAAAAAAC  yGTCCCCGTC
151 TGGCACAAAG  ACGTGCGCTA  TTkTGAATTG  CAACAAACG   GCGAAmCCAT
201 AGGCGGCGTT  TATATGGATT  TGTACGCACG  CGAAGGCAAA  CGCGGCGCG
251 CGTGGATGAA  CGACTACAAA  GGCCGCCGCC  GTTTTTCAGA  CGGCACGCTG
301 CAAyTGCCCA  CCGCCTACCT  CGTCTGCAAC  TTCGCCCCAC  CCGTCGGCGG
351 CAGGGAAGCC  CGCyTGAGCC  ACGACGAAAT  CCTCATCCTC  TTCCACGAAA
401 CCGGACACGG  GCTGCACCAC  CTGCTTACCC  AAGTGGACGA  ACTGGGCGTA
451 TCCGGCATCA  ACGGCGTAKA  ATGGGACGCG  GTCGAACTGC  CCAGCCAGTT
501 TATGGAAAAT  TTCGTTTGGG  AATACAATGT  CTTGGCACA   mTGTCAGCCC
551 ACGAAGAAAC  CGGcgTTCCC  yTGCCGAAAG  AACTCTTsGA  CAAAwTGCTC
601 GCCGCCAAAA  ACTTCCAAsG  CGGCATGTTT  yTsGTCCGGC  AAwTGGAGTT
651 CGCCCTCTTT  GATATGATGA  TTTACAGCGA  AGACGACGAA  GGCCGTCTGA
701 AAAACTGGCA  ACAGGTTTTA  GACAGCGTGC  GCAAAAAAGT  CGCCGTCATC
751 CAGCCGCCCG  AATACAACCG  CTTGCGCTTG  AGCTTCGGCC  ACATCTTCGC
801 AGGCGGCTAT  TCCGAGCTn  ATTACAGCTA  CGCGTGGGCG  GAAGTATTGA
851 GCGCGGACGC  ATACGCCGCC  TTTGAAGAAA  GCGACGATGT  CGCCGCCACA
901 GGCAAACGCT  TTTGGCAGGA  AATCCTCGCC  GTCGGGgnAT  CGCGCAGCGG
951 nGCAGAATCC  TTCAAAGCCT  TCCGCGGCCG  CGAACCGAGC  ATAGACGCAC
1001 TCTTGCGCCA  CAGCGGTTTC  GACAACGCGG  TCTGA

```

This corresponds to the amino acid sequence <SEQ ID 51; ORF 128>:

m128.pep (partial)

```

1  MTDNALLHLG  EEPRFDQIKT  EDIKPALQTA  IAEAREQIAA  IKAQTHTGWA
51  NTVEPLTGIT  ERVGRIWGVV  SHLNCVADTP  ELRAVYNELM  PEITVFFTEI
101 GQDIELYNRF  KTIKNSPEFD  TLSPAQKTKL  NH
//
1  YASEKLREAK  YAFSETXVKK  YFPVGXVLNG  LFAQXKKLYG  IGFTEKTPV

```

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```

51  WHKDVRYXEL QQNGEXIGGV YMDLYAREGK RGGAWMNDYK GRRRFSDGTL
101 QLPTAYLVCN FAPPVGGREA RLSHDEILIL FHETGHGLHH LLTQVDELGV
151 SGINGVXWDA VELPSQFMEN FVWEYNVLAQ XSAHEETGVP LPKELXDKXL
201 AAKNFQXGMF XVRQXEFALF DMMIYSEDDE GRLKNWQQVL DSVRKVKVAVI
251 QPPEYNRFAL SFGHIFAGGY SAAKXSYAWA EVLSADAYAA FEESDDVAAT
301 GKRFWQEILA VGXSRSGAES FKAFRGREPS IDALLRHSGF DNAV*

```

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 52>:

```

g128.seq
1  atgattgaca acgCActgct ccacttgggc gaagaaccCC GTTTaatca
51  aatccaaacc gaagACatca AACCCGCCGT CCAAACCGCC ATCGCCGAAG
101 CGCGCGGACA AATCGCCGCC GTCAAAGCGC AAACGCACAC CGGCTGGGCG
151 AACACCGTCG AGCGTCTGAC CGGCATCACC GAACGCGTCG GCAGGATTTG
201 GGGCGTCGTG TCCCATCTCA ACTCGTCGTG CGACACGCCC GAACTGCGCG
251 CCGTCTATAA CGAACTGATG CCTGAAATCA CCGTCTTCTT CACCGAAATC
301 GGACAAGACA TCGAACTGTA CAACCGCTTC AAAACCATCA AAAATTCCCC
351 CGAATTGCA ACGCTTTCCC CCGCACAAAA AACCAAGCTC GATCAGCACC
401 TGCGCGATTT CGTATTGAGC GGCGCGGAAC TGCCGCCCGA ACGGCAGGCA
451 GAACTGGCAA AACTGCAAAC CGAAGGCGCG CAACTTTCGG CCAAATTCTC
501 CCAAACGCTC CTAGACGCGA CCGACGCGTT CGGCATTAC TTTGACGATG
551 CCGCACCGCT TGCCGGCATT CCCGAAGACG CGCTCGCCAT GTTTGCCGCC
601 GCCGCGCAAA GCGAAGGCAA AACAGGTTAC AAAATCGGCT TGCGATTCC
651 GCACTACCTT GCCGTTATCC AATACGCCGG CAACCGCGAA CTGCGCGAAC
701 AAATCTACCG CGCCTACGTT ACCCGTGCCA GCGAACTTTC AAACGACGGC
751 AAATTCGACA ACACCGCCAA CATCGACCGC ACGCTCGAAA ACGCATTGAA
801 AACCGccaaa cTGCTCGGCT TTAATAATTA CGCCGAATTG TCGCTGGCAA
851 CCAAATGGC GGACACGCCC GAACAGGTTT TAAACTTCCT GCACGACCTC
901 GCCCGCGCGC CCAAACCCTA CGCCGAAAAA GACCTCGCCG AAGTCAAAGC
951 CTTGCGCCGC GAACACCTCG GTCTCGCCGA CCCGCAGCCG TGGGACTTGA
1001 GCTACGCCCG CGAAAACTG CGCGAAGCCA AATACGCATT CAGCGAAACC
1051 GAAGTCAAAA AATACTTCCC CGTCGGCAAA GTTCTGGCAG GCCTGTTCGC
1101 CAAAATCAAA AAATCTACG GCATCGGATT CGCCGAAAAA ACCGTTCCCG
1151 TCTGGCACAA AGACGTGCGC TATTTTGAAT TGCAACAAAA CGGCAAAACC
1201 ATCGCGGCGC TTTATATGGA TTGTACGCA CGCGAAGGCA AACGCGGCGG
1251 CGCGTGGAAT AACGActaca AAGGCCGCCG CCGCTTTGCC GACGgcacGC
1301 TGCAACTGCC CACCGCTAC CTGCTCTGCA ACTTCGCCCC GCCCGTCGGC
1351 GGCAAAGAAG CGCGTTTAAG CCACGACGAA ATCCTCACCC TCTTCCACGA
1401 AacCGGCCAC GGAATGCACC ACCTGCTTAC CCAAGTGGAC GAACTGGGCG
1451 TGTCCGGCAT CAacggcgtA GAATGGGACG CGGTCGAACT GCCCAGCCAG
1501 TTTATGGAAG ACTTCGTTTG GGAATACAAT GTATTGGCAC AAATGTCCCG
1551 CCACGAAGAA AccgGCGAGC CCCTGCCGAA AGAACTCTTC GACAAAATGC
1601 TcgcCGCCAA AAATTTCCAG CGCGGTATGT TCCTCGTCCG GCAAATGGAG
1651 TTCGCCCTCT TCGATATGAT GATTTACAGT GAAAGCGACG AATGCCGTCT
1701 GAAAAACTGG CAGCAGGTTT TAGACAGCGT GCGCAAAGAA GtcGCCGTCA
1751 TCCAACCGCC CGAATACAAC CGCTTCGCCA ACAGCTTCGG CCacatctTC
1801 GCcggcGGCT ATTCCGCAGG CTATTACAGC TACGCATGGG CCGAAGTCct
1851 cAGCACCGAT GCCTACGCCG CCTTTGAAGA AAGcGACGac gtcGCCGCCA
1901 CAGGCAAACG CTTCTGGCAA GAAAtccttg ccgtcggcgg ctCCCGCAGC
1951 gcgCGGAAT CTTTCAAAGC CTTCGCGGGA CGCGAACCGA GCATAGACGC
2001 ACTGCTGCGC CAAagcggT TCGACAACGC gGcttgA

```

This corresponds to the amino acid sequence <SEQ ID 53; ORF 128.ng>:

```

g128.pep
1  MIDNALLHLG EEPFRNQIQT EDIKPAVQTA IAEARGQIAA VKAQHTGWa
51  NTVRLTGIT ERVGRIWGVV SHLNSVVDTP ELRAVYNELM PEITVFFTEI
101 GQDIELYNRF KTIKNSPEFA TLSPAQKTKL DHDLRDFVLS GAELPPERQA
151 ELAKLQTEGA QLSAKFSQNV LDATDAFGIY FDDAAPLAGI PEDALAMFAA
201 AAQSEGKTGY KIGLQIPHYL AVIQYAGNRE LREQIYRAYV TRASELSNDG

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```

251 KFDNTANIDR TLENALKTAK LLGFKNYAEL SLATKMADTP EQVLNPLHDL
301 ARRAKPYAEK DLAEVKAFAR EHLGLADPQP WDSL YAGEKL REAKYAFSET
351 EVKKYFPVVGK VLAGLFAQIK KLYGIGFAEK TVPVWHKDVR YFELQONGKT
401 IGGVYMDLYA REGKRGGAWM NDYKGRRRFA DGTQLQPTAY LVCNFAPPVG
451 GKEARLSHDE ILTLFHETGH GLHLLTQVD ELGVSGINGV EWD AVELPSQ
501 FMENFVWEYN VLAQMSAHEE TGEPLPKELF DKMLAAKNFQ RGMFLVRQME
551 FALFDDMIYS ESDECRLKNW QQVLDVSRKE VAVIQPPEYN RFANSFGHIF
601 AGGYSAGYYS YAWAEVLSTD AYA AFEESDD VAATGKRFWQ EILAVGGSRS
651 AAESFKAFRG REPSIDALLR QSGFDNAA*

```

ORF 128 shows 91.7% identity over a 475 aa overlap with a predicted ORF (ORF 128.ng)
from *N. gonorrhoeae*:

m128/g128

```

          10      20      30      40      50      60
g128.pep  MIDNALLHLGEEPRFNQIQTEDIKPAVQTAIAEARGQIAAVKAQHTGWANTVERLTGIT
          |||||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||
m128      MTDNALLHLGEEPRFDQIKTEDIKPALQTAIAEAREQIAAIIKAQHTGWANTVEPLTGIT
          10      20      30      40      50      60

          70      80      90     100     110     120
g128.pep  ERVGRIGVVSHLSNVVDTPELRAVYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFA
          |||||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||
m128      ERVGRIGVVSHLNCVADTPELRAVYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFD
          70      80      90     100     110     120

          130     140     150     160     170     180
g128.pep  TLSPAQKTKLDHDLRDFVLSGAELPPERQAELAKLQTEGAQLSAKFSQNVLDATDAFGIY
          |||||||:|
m128      TLSPAQKTKLNH
          130
                //

                                340     350     360
g128.pep  YAGEKLREAKYAFSETEVKKYFPVGKVLG
                                ||:|||||:|||||:|||||:|
m128      YASEKLREAKYAFSETXVKKYFPVGXVLNG
                                10      20      30

          370     380     390     400     410     420
g128.pep  LFAQIKKLYGIGFAEKTVPVWHKDVRYPFELQONGKTIIGGVYMDLYAREGKRGGAWMNDYK
          |||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||
m128      LFAQXKKLYGIGFTEKTVPVWHKDVRYPXELQONGEXIGGVYMDLYAREGKRGGAWMNDYK
          40      50      60      70      80      90

          430     440     450     460     470     480
g128.pep  GRRRFADGTLQLPTAYLVCNFAPPVGGKEARLSHDEILTLFHETGHGLHLLTQVDELGV
          |||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||
m128      GRRRFSDGTLQLPTAYLVCNFAPPVGGREARLSHDEILILFHETGHGLHLLTQVDELGV
          100     110     120     130     140     150

          490     500     510     520     530     540
g128.pep  SGINGVEWDAVELPSQFMENFVWEYNVLAQMSAHEETGEPLPKELFDKMLAAKNFQXGMF
          |||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||
m128      SGINGVXWDAVELPSQFMENFVWEYNVLAQXSAHEETGVPLPKELXDKMLAAKNFQXGMF
          160     170     180     190     200     210

          550     560     570     580     590     600

```

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```

g128.pep      LVRQMEFALFDDMMIYSESDCRLKNWQQVLDSVRKEVAVIQPPEYNRFANSFGHIFAGGY
                |||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
m128          XVRQXEFALFDDMMIYSEDDGRLKNWQQVLDSVRKKVAVIQPPEYNRFALSFGHIFAGGY
                220      230      240      250      260      270

                610      620      630      640      650      660
g128.pep      SAGYYSYAWAEVLSTDAYAAFEESDDVAATGKRFWQEILAVGGSRSAAESFKAFRGREPS
                |||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
m128          SAAXYSYAWAEVLSADAYAAFEESDDVAATGKRFWQEILAVGXSRSGAESFKAFRGREPS
                280      290      300      310      320      330

                670      679
g128.pep      IDALLRQSGFDNAAX
                |||:|||||:|||||:
m128          IDALLRHSGFDNAVX
                340

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 54>:

```

a128.seq
1   ATGACTGACA  ACGCACTGCT  CCATTTGGGC  GAAGAACCCC  GTTTTGATCA
51  AATCAAAACC  GAAGACATCA  AACCCTGCCCT  GCAAACCCGCC  ATTGCCGAAG
101 CGCGCGAACA  AATCGCCGCC  ATCAAAGCCC  AAACGCACAC  CGGTGGGCA
151 AACACTGTCG  AACCCCTGAC  CGGCATCACC  GAACGCGTCG  GCAGGATTTG
201 GGGCGTGGTG  TCGCACCTCA  ACTCCGTAC  CGACACGCCC  GAACTCGCGC
251 CGCCTACAA  TGAATTAATG  CCGGAATTA  CCGCTTCTTT  CACCGAAATC
301 GGACAAGACA  TCGAGCTGTA  CAACCGCTTC  AAAACCATCA  AAAACTCCCC
351 CGAGTTCGAC  ACCTCTCCCC  ACGCGCAAAA  AACCAAATC  AACCAGCATC
401 TGGCGGATTT  TGCCTCTAGC  GGC CGGGAAC  TGCCCGCCGA  ACAGCAGGCA
451 GAATTGGCAA  AACTGCAAAC  CGAAGCGCGC  CAACTTTCCG  CCAAAATCTC
501 CCAAAACGTC  CTAGACGCGA  CCGACGCGTT  CGGCATTTC  TTTGACGATG
551 CCGCACCGCT  TGCCGGCATT  CCGCAAGACG  CGCTCGCCAT  GTTTCGCCCT
601 GCGCGCAAAA  GCGGAAGCAA  AACAGGCTAC  AAAATCGGTT  TGCAGATTCC
651 GCACTACCTC  GCCGTATCC  AATACGCCGA  CAACCGCAAA  CTGCGCGAAC
701 AAATCTACCG  CGCCTACGTT  ACCCGCGCCA  GCGAGCTTTC  AGACGACGGC
751 AAATTGACA  ACACCGCCAA  CATCGACCGC  ACGCTCGAAA  ACGCCCTGCA
801 AACCGCCAAA  CTGCTCGGCT  TCAAAAACTA  CGCGGAATTG  TCGCTGGCAA
851 CCAAAATGGC  GGACACCCCC  GAACAAGTTT  TAAATCTCT  GCACGACCTC
901 GCCCGCCGGC  CCAAACCTTA  CGCCGAAAAA  GACCTCGCCG  AAGTCAAAAG
951 CTTGCGCCGC  GGAAGCCTCG  GCCTCGCCGA  TTTGCAACCG  TGGGACTTGG
1001 GCTACGCCGG  CGAAAAACTG  CGCGAAGCCA  AATACGCATT  CAGCGAAACC
1051 GAAATCAAAA  AATACTTCCC  CGTCGCGAAA  GTATTAACG  GACTGTTCCG
1101 CCAATCAAAA  AAACCTCTAGC  GCATCGGAAT  TACCGAAAAA  ACCGTCCTCC
1151 TCTGGACAAA  AGACGTGCGC  TATTTTGAAT  TGCACAAAAA  CGGCGAAACC
1201 ATAGCGCGCG  TTTATATGGA  TTTGTACGCA  CGCGAAGGCA  AACCGGCGCG
1251 CGCGTGGATG  AACGACTACA  AAGGCGCGCT  CGGTTTTTCA  GCGGCGACGC
1301 TGCAACTGCC  CACCGCTAC  CTCGTGCGA  ACTTCACCCC  GACCGCTCGC
1351 GGCAAAGAAG  CCCGCTTGAG  CCATGACGAA  ATCCTCACCC  TCTTCCACGA
1401 AACCGGCAC  GGCCTGCACC  ACCTGCTTAC  CCAAGTCGAC  GAACTGGGGC
1451 TATCCGGCAT  CAACGGCGTA  GAATGGGACG  CAGTCGAAT  CCCCAGTCAG
1501 TTTATGGAAA  ATTTCTGTTG  GGAATACAAT  GTCTTGCGCG  AAATGTCCGC
1551 CCACGAAGAA  ACCGGCGTTC  CCTGCGCGAA  AGAATCTCTC  GACAAAATGC
1601 TCGCGCCCAA  AAATCTCCAA  CGCGGAATGT  TCCTCGTCCG  CCAAAATGGG
1651 TTCGCGCTCT  TTGATATGAT  GATTACAGC  GAAGACGACG  AAGCGCGTCT
1701 GAAAACTGG  CAACAGGTTT  TAGACAGCGT  GCGCAAAGAA  GTCGCCGTCG
1751 TCCGACCGCC  CGAATACAA  CGCTTCGCCA  ACAGCTTCGG  CCGCATCTTC
1801 GCAGCGGCT  ATTCGCGAG  CTATTACAGC  TACGCGTGGG  CGGAAGTATT
1851 GAGCGCGGAC  GCATACGCG  CCTTTGAAGA  AAGCGACGAT  GTCGCCGCCA
1901 CAGGCAAAAC  CTTTTGGCAG  GAAATCCTCG  CCGTCGGCGG  ATCGCGCAGC
1951 GCGGCAGAAT  CTTTCAAAGC  CTTCCGCGGA  CGCGAACCGA  GCATAGACGC
2001 ACTCTTGGC  CACAGCGGCT  TGCACAACGC  GGCTTGA

```

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This corresponds to the amino acid sequence <SEQ ID 55; ORF 128.a>:

```

a128.pep
  1 MTDNALLHLG EEPFRDQIKT EDIKPALQTA IAEAREQIAA IKAQHTGTWA
 51 NTVEPLTGIT ERVGRIWGVV SHLNSVTDTP ELRAAYNELM PEITVFFTEI
101 GQDIELYNRF KTIKNSPEFD TLSHAQKTKL NHDLRDFVLS GAELPPEQQA
151 ELAKLQTEGA QLSAKFSQNV LDATDAFGIY FDDAAPLAGI PEDALAMFAA
201 AAQSEGKTGY KIGLQIPHYL AVIQYADNRK LREQIYRAYV TRASELSDDG
251 KFDNTANIDR TLENALQTAK LLGFKNYAEL SLATKMADTP EQVLNFLHDL
301 ARRAKPYAEK DLAEVKAFAR ESLGLADLQP WDLGYAGEKL REAKYAFSET
351 EVKKYFPVGK VLNGLFAQIK KLYGIGFTEK TVPVVHKDVR YFELQONGET
401 IGGVYMDLYA REGKRGGAWM NDYKGRRRFS DGTLLQLPTAY LVCNFTFPVG
451 GKEARLSHDE ILTLFHETGH GLHLLTQVD ELGVSGINGV EWDAVELPSQ
501 FMENFVWEYN VLAQMSAHEE TGVPLPKELF DKMLAAKNFQ RGMFLVRQME
551 FALFDMMIYS EDDEGRLEKNW QQVLDSVRKE VAVVRPEYN RFANSFGHIF
601 AGGYSAGYYS YAWAEVLSAD AYAAFEESDD VAATGKRFWQ EILAVGGSRS
651 AAESFKAFRG REPSIDALLR HSGFDNAA*

```

m128/a128 ORFs 128 and 128.a showed a 66.0% identity in 677 aa overlap

```

m128.pep      10      20      30      40      50      60
MTDNALLHLGEEPRFDQIKTEDIKPALQTAIAEAREQIAAIIKAQHTGTWANTVEPLTGIT
|||||
a128          10      20      30      40      50      60
MTDNALLHLGEEPRFDQIKTEDIKPALQTAIAEAREQIAAIIKAQHTGTWANTVEPLTGIT

m128.pep      70      80      90      100     110     120
ERVGRIWGVVSHLNCVADTPELRAVYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFD
|||||
a128          70      80      90      100     110     120
ERVGRIWGVVSHLNSVTDTPELRAAYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFD

m128.pep      130
TLSPAQKTKLNH-----
|||
a128          130     140     150     160     170     180
TLSHAQKTKLNHDLRDFVLSGAELPPEQQAELAKLQTEGAQLSAKFSQNVLDATDAFGIY

m128.pep      -----
a128          190     200     210     220     230     240
FDDAAPLAGIPEDALAMFAAAAQSEGKTGYKIGLQIPHYLAVIQYADNRKLREQIYRAYV

m128.pep      -----
a128          250     260     270     280     290     300
TRASELSDDGKFDNTANIDRTLENALQTAKLLGFKNYAELSLATKMADTP EQVLNFLHDL

m128.pep      -----
a128          310     320     330     340     350     360
ARRAKPYAEKDLAEVKAFARESLGLADLQPWDLGYAGEKLREAKYAFSETEVKYFPVGK

m128.pep      160     170     180     190     200     210
VLNGLFAQXKKLYGIGFTEKTVPVVHKDVRYXELQONGEXIGGVYMDLYAREGKRGGAWM
|||||
a128          370     380     390     400     410     420
VLNGLFAQIKKLYGIGFTEKTVPVVHKDVRYFELQONGETIGGVYMDLYAREGKRGGAWM

m128.pep      220     230     240     250     260     270
NDYKGRRRFS DGTLLQLPTAYLVCNFAPPVGGREARLSHDEILILFHETGHGLHLLTQVD

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a128      NDYKGRRRFSDGTLQLPTAYLVCNFTFPVGGKEARLSHDEILTLFHETGHGLHLLTQVD
          430          440          450          460          470          480

m128.pep  280          290          300          310          320          330
          ELGVSGINGVXWDAVELPSQFMENFVWEYNVLAQXSAHEETGVPLPKELXDKXLAAKNFQ
          |||||
a128      ELGVSGINGVEWDAVELPSQFMENFVWEYNVLAQMSAHEETGVPLPKELFDKMLAAKNFQ
          490          500          510          520          530          540

m128.pep  340          350          360          370          380          390
          XGMFXVRQXEFALFDDMMIYSEDDEGR LKNWQQVLD SVRKKVAVIQPPEYNRFALSFGHIF
          |||
a128      RGMFLVRQMEFALFDDMMIYSEDDEGR LKNWQQVLD SVRKEVAVVRPPEYNRFANSFGHIF
          550          560          570          580          590          600

m128.pep  400          410          420          430          440          450
          AGGYSAA XSYAWAEVLSADAYAAFEESDDVAATGKRFWQEILAVGXSRSGAESFKAFRG
          |||||:
a128      AGGYSAGYYSAWAEVLSADAYAAFEESDDVAATGKRFWQEILAVGGSRSAAESFKAFRG
          610          620          630          640          650          660

m128.pep  460          470
          REPSIDALLRHSGFDNAVX
          |||||:
a128      REPSIDALLRHSGFDNAAX
          670

```

Further work revealed the DNA sequence identified in *N. meningitidis* <SEQ ID 56>:

```

ml28-1.seq
1  ATGACTGACA  ACGCACTGCT  CCAATTGGGC  GAAGAACCCC  GTTTTGATCA
51  AATCAAAACC  GAAGACATCA  AACCCCGCCCT  GCAAACCGCC  ATGCGCCGAAG
101 CGCGCGGAACA  GAATCGCCGCC  ATCAAAGCCC  AAACCGCACAC  CGCTGGGGCA
151 AACACTGTCTG  AACCCCTGAC  CGGCATCACC  GAACGCGTCG  GCAGGATTTG
201 GGGCGTGGTG  TCGCACTCA  ACTCGTCTCG  CGACACGCCC  GAACTCGCGC
251 CGCTCTATAA  CGACTGATG  CCCGAAATCA  CCGTCTTCTT  CACCGAAATC
301 GGACAAGACA  TCGAGCTGTA  CAACCGCTTC  AAAACCATCA  AAAATTCCCC
351 CGAATTCGAC  ACCCTCTCCC  CCGCACAAAA  AACCAAACTC  AACCACGATC
401 TGCGCGGATT  CGTCTCTAGC  GGGCGCGGAAC  TGCCGCCCGA  ACAGCAGGCA
451 GAACTGGCAA  AACTGCAAA  CGAAGGCGCG  CAACTTTCG  CCAAAATCTC
501 CCAAAACGTC  CTAGACGCGA  CCGACGCGTT  CGGCAATTAC  TTTGACGATG
551 CCGCACCCTG  TGCCGGCAT  CCCGAAGACG  CGCTCGCCAT  GTTTGCCGCC
601 GCCGCGCAAA  GCGAAAGCAA  AACAGGCTAC  AAAATCGGCT  TGCAGATTCC
651 ACACCTACCT  CGCGTCTACC  AATACCGCGA  CAACCGCGAA  TGCGCGGAAC
701 AAATCTACCG  CGCCTACGTT  ACCCGCGCCA  GCGAACTTTC  AGACGACGGC
751 AAATTCGACA  ACACCGCCAA  CATCGACCGC  ACGCTCGCAA  ACGCCCTGCA
801 AACCGCAAA  CTGCTCGGCT  TCAAAAAC  CGCGGAATTG  TCGCTGGCAA
851 CCAAAATGTC  GGACGCGCCC  GAACAAGTTT  TAAACTTCCT  GCACGACCTC
901 GCCCGCCGCG  CCAAAACCTA  CGCGGAAAA  GACCTCGCCG  AAGTCAAAGC
951 CTTCCGCCCG  GAAAGCCTGA  ACCTCGCCGA  TTTGCAACCG  TGGGACTTGG
1001 GCTACGCCAG  CGAAAAC  CGCGAAGCCA  AATACCGGTT  CAGCGAAACC
1051 GAAGTCAAAA  AATACTTCCC  CGTCGGCAA  GTATTAAACG  GACTTCTCGC
1101 CCAAAATCAA  AAATCTACG  GCATCGGATT  TACCGAAAA  ACCGTCCCCG
1151 TCTGGCACA  AGACGTGCGC  TATTTTGAAT  TGCACAAAA  CGGCGAAACC
1201 ATAGGCGCGC  TTTATATGA  TTTGTACGA  CGCGAAGGCA  AACGCGCGCG
1251 CGCGTGGATG  AACGACTACA  AAGGCCGCGC  CCGTTTTTCA  GACGGCAGCG
1301 TGCAACTGAC  CACCGCTAC  CTGCTCTGCA  ACTTCGCCCC  ACCGCTCGCG
1351 GCGAGGGAAC  CCCGCTGAG  CCACGACGAA  ATCCTCATCC  TCTTCCACGA
1401 AACCGGACAC  GGGCTGAC  ACCTGCTTAC  CCAAGTGGAC  GAACTGGGCC

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1451 TATCCGGCAT CAACGGCGTA GAATGGGACG CGGTGGAAC GCCCAGCCAG
1501 TTTATGGAAA ATTTCTGTTT GGAATACAAT GTCTTGGCAC AAATGTCAGC
1551 CCACGAAGAA ACCGGCGTTC CCCTGCCGAA AGAACTCTTC GACAAAATGC
1601 TCGCCGCCAA AAACCTCCAA CGCGGCATGT TCCTCGTCCG GCAAATGGAG
1651 TTCGCCCTCT TTGATATGAT GATTACAGC GAAGACGACG AAGGCCGTCT
1701 GAAAACTGG CAACAGGTTT TAGACAGCGT GCGCAAAAAA GTCGCCGTCA
1751 TCAGCCGCC CGAATACAAC CGCTTCGCCT TGAGCTTCGG CCACATCTTC
1801 GCAGGCGGCT ATTCCGCGAG CTATTACAGC TACGCGTGGG CGGAAGTATT
1851 GAGCGCGGAC GCATACGCCG CCTTTGAAGA AAGCGACGAT GTCGCCGCCA
1901 CAGGCAAACG CTTTGGGAG GAAATCCTCG CCGTCGGCGG ATCGCGCAGC
1951 GCGGCAGAA CTTCAAAGC CTTCCGCGGC CGCAACCGA GCATAGACGC
2001 ACTCTTGGC CACAGCGGTT TCGACAACGC GGTCTGA

```

This corresponds to the amino acid sequence <SEQ ID 57; ORF 128-1>:

m128-1.pep.

```

1 MTDNALLHLG EEPFRDQIKT EDIKPALQTA IAEAREQIAA IKAQTHTGWA
51 NTVEPLTGIT ERVGRIWGVV SHLSNVADTP ELRAVYNELM PEITVFTEI
101 GQDIELYNRF KTIKNSPEFD TLSPAQKTKL NHDLRDFVLS GAELPPEQQA
151 ELAKLQTEGA QLSAKFSQNV LDATDAFGIY FDDAAPLAGI PEDALAMFAA
201 AAQSESKTGY KIGLQIPHYL AVIQYADNRE LREQIYRAYV TRASELSDDG
251 KFDNTANIDR TLANALQTAK LLGFKNYAEL SLATKMADTP EQVLNLFHDL
301 ARRAKPYAEK DLAEVKAFAR ESLNLADLQP WDLGYASEKL REAKYAFSET
351 EVKKYFPVKG VINGLFAQIK KLYGIGFTEK TVPVVHKDVR YFELQONGET
401 IGGVYMDLYA REGKRGGAWM NDYKGRRRFS DGTLLQPTAY LVCNFAPPVG
451 GREARLSHDE ILILFHETGH GLHLLTQVD ELGVSGINGV EWDAVELPSQ
501 FMENFVWEYN VLAQMSAHEE TGVPLPKELF DKMLAAKNFQ RGMFLVRQME
551 FALFDMMIYS EDEGRLLKNW QVLDVSRKK VAVIQPPEYN RFALSFGHIF
601 AGGYSAGYYS YAWAEVLSAD AYAAFEESDD VAATGKRFWQ EILAVGGSRS
651 AAESFKAFRG REPSIDALLR HSGFDNAV*

```

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 58>:

g128-1.seq (partial)

```

1 ATGATTGACA ACGCACTGCT CCACTTGGGC GAAGAACCCC GTTTTAATCA
51 AATCAAAACC GAAGACATCA AACC CGCGT CCAAACCGCC ATCGCCGAAG
101 CGCGCGGACA AATCGCGGCC GTCAAAGCGC AAACGCACAC CGGCTGGGCG
151 AACACCGTCG AGCGTCTGAC CGGCATCACC GAACCGGTCG GCAGGATTTG
201 GGGCGTCGTG TCCCATCTCA ACTCCGTCGT CGACACGCCC GAACTGCGCG
251 CCGTCTATAA CGAACTGATG CCTGAAATCA CCGTCTCTT CACCGAAATC
301 GGACAAGACA TCGAACTGTA CAACCGCTTC AAAACCATCA AAAATTCCCC
351 CGAATTGACA ACGCTTTCCC CCGCACAAAA AACCAGCTC GATCACGACC
401 TGCGCGATTT CGTATTGAGC GCGCGGGAAC TGCCGCCCGA ACGGCAGGCA
451 AACTGGCAA AACTGCAAAAC CGAAGGCGCG CAACTTTCCG CCAAAATCTC
501 CCAAACGTC CTAGACGCGA CCGACGCGTT CGGCATTTC TTTGACGATG
551 CCGCACCGCT TGCCGGCATT CCCGAAGACG CGCTCGCCAT GTTTCGCGCC
601 GCGCGGCAAA GCGAAGGCAA AACAGGTTAC AAAATCGGCT TGCAGATTCC
651 GCACTACCTT GCCGTTATCC AATACGCCG CAACCGCGAA CTGCGCGAAC
701 AAATCTACCG CGCCTACGTT ACCCGTGCCA GCGAATTTT AAACGACGGC
751 AAATTCGACA ACACCGCAA CATCGACCGC ACGTCGAAA ACGCATTGAA
801 AACCGCCAAA CTGCTCGGCT TAAAAAATTA CGCCGAATTG TCGCTGGCAA
851 CCAAATGGC GGACACGCCC GAACAGGTTT TAAACTTCCT GCACGACCTC
901 GCGCGCGCG CCAAACCTTA CGCCGAAAAA GACCTCGCCG AAGTCAAAGC
951 CTTGCGCCG GAACACCTCG GTCTCGCCGA CCCGACGCG TGGGACTTGA
1001 GCTACGCCG CGAAAACTG CGCGAAGCCA AATACGCATT CAGCGAAACC
1051 GAAGTCAAAA AATACTTCCC CGTCGGCAA GTTCTGGCAG GCCTGTTCCG
1101 CCAAATCAA AAACCTACG GCATCGGATT CGCCGAAAA ACCGTTCCCG
1151 TCTGGCACA AGACGTGCGC TATTTTGAAT TGCAACAAA CGGCAAAACC
1201 ATCGGCGCG TTTATATGGA TTTGTACGCA CGCGAAGGCA AACGCGGCGG
1251 CGCGTGGATG AACGACTACA AAGCGCGCG CCGCTTTGCC GACGGCACGC
1301 TGCAACTGCC CACCGCTAC CTGCTCTGCA ACTTCGCCCC GCCCGTCGGC
1351 GGCAAAGAAG CGCGTTTAAG CCACGACGAA ATCCTACCC TCTTCCACGA
1401 AACCGGCCAC GGAATGCACC ACCTGCTTAC CCAAGTGGAC GAACTGGGCG
1451 TGTCGGCAT CAACGGCGTA AAA

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This corresponds to the amino acid sequence <SEQ ID 59; ORF 128-1.ng>:

g128-1.pep (partial)

```

1  MIDNALLHLG EEPRENOIKT EDIKPAVQTA IAEARGQIAA VKAQHTGWGA
51  NTVRLTGIT ERVGRIWGVV SHLNSVVDTP ELRAVYNELM PEITVFFTEI
101 GQDIELYNRF KTIKNSPEFA TLSPAQKTKL DHDLRDFVLS GAELPPERQA
151 ELAKLQTEGA QLSAKFSQNV LDATDAFGIY FDDAAPLAGI PEDALAMFAA
201 AAQSEGKTGY KIGLQIPHYL AVIQYAGNRE LREQIYRAYV TRASELSNDG
251 KFDNTANIDR TLENALKTAK LLGFKNYAEL SLATKMADTP EQVLNLFHDL
301 ARRAKPYAEK DLAEVKAFAR EHLGLADPQP WDLSYAGEKL REAKYAFSET
351 EVKKYFPVGK VLAGLFAQIK KLYGIGFAEK TVPVWHKDVR YFELQONGKT
401 IGGVYMDLYA REGKRGGAWM NDYKGRRRFA DGTQLQLPTAY LVCNFAPPVG
451 GKEARLSHDE ILTLFHETGH GLHLLTQVD ELGVSGINGV K

```

m128-1/g128-1 ORFs 128-1 and 128-1.ng showed a 94.5% identity in 491 aa overlap

g128-1.pep	10	20	30	40	50	60
	MIDNALLHLGEEPRFNQIKTEDIKPAVQTAIAEARGQIAAVKAQHTGWANTVERLTGIT					
m128-1	10	20	30	40	50	60
	MTDNALLHLGEEPRFDQIKTEDIKPALQTAIAEAREQIAAIAQHTGWANTVEPLTGIT					
g128-1.pep	70	80	90	100	110	120
	ERVGRIWGVVSHLNSVVDTPPELRVYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFA					
m128-1	70	80	90	100	110	120
	ERVGRIWGVVSHLNSVADTPPELRVYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFD					
g128-1.pep	130	140	150	160	170	180
	TLSPAQKTKLDHDLRDFVLSGAELPPERQAEELAKLQTEGAQLSAKFSQNVLDATDAFGIY					
m128-1	130	140	150	160	170	180
	TLSPAQKTKLNHDLRDFVLSGAELPPEQQAEELAKLQTEGAQLSAKFSQNVLDATDAFGIY					
g128-1.pep	190	200	210	220	230	240
	FDDAAPLAGIPEDALAMFAAAQSEGKTGYKIGLQIPHYLAVIQYAGNRELREQIYRAYV					
m128-1	190	200	210	220	230	240
	FDDAAPLAGIPEDALAMFAAAQSESKTGYKIGLQIPHYLAVIQYADNRELREQIYRAYV					
g128-1.pep	250	260	270	280	290	300
	TRASELSNDGKFDNTANIDRTLENALKTAKLLGFKNYAELSLATKMADTPEQVLNLFHDL					
m128-1	250	260	270	280	290	300
	TRASELSDDGKFDNTANIDRTLANALQTAKLLGFKNYAELSLATKMADTPEQVLNLFHDL					
g128-1.pep	310	320	330	340	350	360
	ARRAKPYAEKDLAEVKAFAREHLGLADPQPWDLSYAGEKLREAKYAFSETEVKKYFPVGK					
m128-1	310	320	330	340	350	360
	ARRAKPYAEKDLAEVKAFARESLNLADLPQWDLGYASEKLREAKYAFSETEVKKYFPVGK					
g128-1.pep	370	380	390	400	410	420
	VLAGLFAQIKKLYGIGFAEKTVPVWHKDVRVFELQONGKTIGGVYMDLYAREGKRGGAWM					
m128-1	370	380	390	400	410	420
	VLNGLFAQIKKLYGIGFTEKTVPVWHKDVRVFELQONGKTIGGVYMDLYAREGKRGGAWM					
g128-1.pep	430	440	450	460	470	480
	NDYKGRRRFADGTQLQLPTAYLVCNFAPPVGGKEARLSHDEILTLFHETGHGLHLLTQVD					

a128-1.pcp

1	MTDNALLHLG	EPRFDQIKT	EDIKPALQTA	IAEAREQIAA	IKAQTHFGWA
51	NTVEPLTGIT	ERVGIWGVV	SHLSVTDTP	ELRAAYNELM	PEITVTFTEI
101	GQDIELYNRF	KTIKNSPEFD	TLSHAQKTKL	NHDLRDFVLS	GAELPPEQQA
151	ELAKLQTEGA	QLSAKFSQNV	LDATADFNGI	FDDAAPLAGI	PEDALAMFAA
201	AAQSEKGTGY	KIGLQIPHYL	AVIQYADNRK	LRQIQYRAYV	TRASELSDGG
251	KFDNTANIDR	TLENALQTAK	LLGFKNYAEL	SLATKMADTP	EQVLNLFHDL

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301  ARRAKPYAEK  DLAEVKAFAR  ESLGLADLQP  WDLGYAGEKL  REAKYAFSET
351  EVKKYFPVGK  VLNLGFAQIK  KLYGIGFTEK  TVPVWHKDVR  YFELQONGET
401  IGGVYMDLYA  REGKRGGAWM  NDYKGRRRFS  DGTLLQLPTAY  LVCNFTPPVG
451  GKEARLSHDE  ILTLFHETGH  GLHLLLTQVD  ELGVSGINGV  EWDAVELPSQ
501  FMENFVWEYN  VLAQMSAHEE  TGVPLPKELF  DKMLAAKNFQ  RGMFLVRQME
551  FALFDMMIYS  EDDEGRLLNW  QQVLDSVRKE  VAVVRPPEYN  RFANSFGHIF
601  AGGYSAGYYS  YAWAEVLSAD  AYAAFEESDD  VAATGKRFWQ  EILAVGGSRS
651  AAESFKAFRG  REPSIDALLR  HSGFDNAA*

```

m128-1/a128-1 ORFs 128-1 and 128-1.a showed a 97.8% identity in 677 aa overlap

a128-1.pep	10	20	30	40	50	60
	MTDNALLHLGEEPRFDQIKTEDIKPALQTAIAEAREQIAAIKAQHTGTWANTVEPLTGIT					
m128-1	MTDNALLHLGEEPRFDQIKTEDIKPALQTAIAEAREQIAAIKAQHTGTWANTVEPLTGIT					
	10	20	30	40	50	60
a128-1.pep	70	80	90	100	110	120
	ERVGRIWGVVSHLNSVTDTPELRAAYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFD					
m128-1	ERVGRIWGVVSHLNSVADTPELRAVYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFD					
	70	80	90	100	110	120
a128-1.pep	130	140	150	160	170	180
	TLSHAQKTKLNHDLRDFVLSGAELPPEQQAELAKLQTEGAQLSAKFSQNVLDATDAFGIY					
m128-1	TLSPAQKTKLNHDLRDFVLSGAELPPEQQAELAKLQTEGAQLSAKFSQNVLDATDAFGIY					
	130	140	150	160	170	180
a128-1.pep	190	200	210	220	230	240
	FDDAAPLAGIPEDALAMFAAAQSEKGTGYKIGLQIPHYLAVIQYADNRKLREQIYRAYV					
m128-1	FDDAAPLAGIPEDALAMFAAAQSEKGTGYKIGLQIPHYLAVIQYADNRELREQIYRAYV					
	190	200	210	220	230	240
a128-1.pep	250	260	270	280	290	300
	TRASELSDDGKFDNTANIDRTLENALQAKLLGFKNYAELSLATKMADTPEQVNLFLHDL					
m128-1	TRASELSDDGKFDNTANIDRTLANALQAKLLGFKNYAELSLATKMADTPEQVNLFLHDL					
	250	260	270	280	290	300
a128-1.pep	310	320	330	340	350	360
	ARRAKPYAEKDLAEVKAFARESLGLADLQPWDLGYAGEKLREAKYAFSETEVKKYFPVGK					
m128-1	ARRAKPYAEKDLAEVKAFARESLNLADLQPWDLGYASEKLREAKYAFSETEVKKYFPVGK					
	310	320	330	340	350	360
a128-1.pep	370	380	390	400	410	420
	VLNLGFAQIKKLYGIGFTEKTVPVWHKDVRVFELQONGETIGGVYMDLYAREGKRGGAWM					
m128-1	VLNLGFAQIKKLYGIGFTEKTVPVWHKDVRVFELQONGETIGGVYMDLYAREGKRGGAWM					
	370	380	390	400	410	420
a128-1.pep	430	440	450	460	470	480
	NDYKGRRRFSDGTLLQLPTAYLVCNFTPPVGGKEARLSHDEILTLFHETGHGLHLLTQVD					
m128-1	NDYKGRRRFSDGTLLQLPTAYLVCNFAPPVGGREARLSHDEILTLFHETGHGLHLLTQVD					
	430	440	450	460	470	480
a128-1.pep	490	500	510	520	530	540
	ELGVSGINGVEWDAVELPSQFMENFVWEYNVLAQMSAHEETGVPLPKELFDKMLAAKNFQ					

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m128-1      ELGVSGINGVEWDAVELPSQFMENFVWEYNVLAQMSAHEETGVPLPKELFDKMLAAKNFQ
              490      500      510      520      530      540
a128-1.pep  RGMFLVRQMEFALFDMMIYSEDDEGR LKNWQQVLD SVRKEVAVVRPPEYNRFANSFGHIF
              550      560      570      580      590      600
m128-1      RGMFLVRQMEFALFDMMIYSEDDEGR LKNWQQVLD SVRKKVAVIQPPEYNRFALSFGHIF
              550      560      570      580      590      600
a128-1.pep  AGGYSAGYYSYAWAEVLSADAYAAFEESDDVAATGKRFWQEILAVGGSRSAAESFKAERG
              610      620      630      640      650      660
m128-1      AGGYSAGYYSYAWAEVLSADAYAAFEESDDVAATGKRFWQEILAVGGSRSAAESFKAERG
              610      620      630      640      650      660
a128-1.pep  REPSIDALLRHSGFDNAAX
              670      679
m128-1      REPSIDALLRHSGFDNAVX
              670

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206

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 62>:

```

m206.seq
1   ATGTTTCCCC CCGACAAAAC CCTTTTCCTC TGTCTCAGCG CACTGCTCCT
51  CGCCTCATGC GGCACGACCT CCGGCAAACA CCGCCAACCG AAACCCAAAC
101 AGACAGTCCG GCAAATCCAA GCCGTCCGCA TCAGCCACAT CGACCGCACA
151 CAAGGCTCGC AGGAATCAT  GCTCCACAGC CTCGGACTCA TCGGCACGCC
201 CTACAAATGG GCGCGCAGCA GCACCGCAAC CGGCTTCGAT TGCAGCGGCA
251 TGATTCAATT CGTTTACAA  AACGCCCTCA ACGTCAAGCT GCCGCGCACC
301 GCCCGCGACA TGGCGGCGGC AAGCCGSA AATCCCCGAC GCCGCTCAA
351 GGCCGCGGAC CTCGTATTCT TCAACACCGG CGGCGCACAC CGTACTCAC
401 ACGTCGGACT CTACATCGGC AACGGCGAAT TCATCCATGC CCCCAGCAGC
451 GGCAAAACCA TCAAAACCGA AAAACTCTCC ACACCGTTT ACGCCAAAAA
501 CTACCTCGGC GCACATACTT TTTTACAGA ATGA

```

This corresponds to the amino acid sequence <SEQ ID 63; ORF 206>:

```

m206.pep..
1   MFPPDKTLFL CLSALLLASC GTTSGKHRQP KPKQTVRQIQ AVRISHIDRT
51  QGSQELMLHS LGLIGTPYKW GGSSTATGFD CSGMIQFVYK NALNVKLPRP
101 ARDMAAASRK IPDSRXKAGD LVFFNTGGAH RYSHVGLYIG NGEFIHAPSS
151 GKTIKTEKLS TPFYAKNYLG AHTFFTE*

```

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 64>:

```

g206.seq
1   atgttttccc cggacaaaac ctttttcttc tgtctcggcg cactgctect
51  cgcctcatgc ggcacgacct ccggcaaaca ccgccaaccc aaacccaaac
101 agacagtccg gcaaattcaa gccgtccgca tcagccacat cggcgcgaca
151 caaggctcgc aggaactcat gctccacagc ctccgactca tcggcacgcc
201 ctacaaatgg ggcggcagca gcaccgcaac cggcttcgac tgcagcggga
251 tgattcaatt ggtttacaaa aacgccctca acgtcaagct gccgcgcacc
301 gcccgcgaca tggcggcggc aagccgcaaa atccccgaca gccgcctcaa
351 ggccggcgac atcgtattct tcaacaccgg cggcgcacac cgctactcac
401 acgtcggact ctacatcggc aacggcgaat tcatccatgc ccccggcagc
451 ggcaaaaacca tcaaaaccga aaaactctcc acaccgtttt acgcaaaaaa
501 ctaccttgga gcgcatacgt tttttacaga atga

```

10 20 30 40 50 60
 m206.pep MFPPDKTLFLCLSALLLASC GTTSGKHRQPKPKQTVRQIQAVRISHIDRTQGSQELMLHS
 |||||
 a206 MFPPDKTLFLCLSALLLASC GTTSGKHRQPKPKQTVRQIQAVRISHIDRTQGSQELMLHS
 10 20 30 40 50 60

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	70	80	90	100	110	120
m206.pep	LGLIGTPYKWGGSSTATGFDCSGMIQFVYKNALNVKLPTARDMAAASRKIPDSRXKAGD					
a206	LGLIGTPYKWGGSSTATGFDCSGMIQFVYKNALNVKLPTARDMAAASRKIPDSRLKAGD					
	70	80	90	100	110	120
	130	140	150	160	170	
m206.pep	LVFFNTGGAHRYSHVGLYIGNGEFIHAPSSGKTIKTEKLSTPFYAKNYLGAHTFFTEX					
a206	LVFFNTGGAHRYSHVGLYIGNGEFIHAPSSGKTIKTEKLSTPFYAKNYLGAHTFFTEX					
	130	140	150	160	170	

287

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 68>:

m287.seq

1	ATGTTTAAAC	GCAGCGTAAT	CGCAATGGCT	TGTATTTTGG	CCCTTTCAGC
51	CTGCGGGGGC	GGCGGTGGCG	GATCGCCCGA	TGTCAAGTCG	GCGGACACGC
101	TGTCAAAACC	TGCCGCCCTT	GTTGTTTCTG	AAAAAGAGAC	AGAGGCCAAAG
151	GAAGATGCGC	CACAGGCAGG	TTCTCAAGGA	CAGGGCGCGC	CATCCGCACA
201	AGGCAGTCAA	GATATGGCGG	CGGTTTCGGA	AGAAAATACA	GGCAATGGCG
251	GTGCGGTAAAC	AGCGGATAAT	CCCAAAAATG	AAGACGAGGT	GGCACAAAAT
301	GATATGCCGC	AAAATGCCGC	CGGTACAGAT	AGTTCGACAC	CGAATCACAC
351	CCCGGATCCG	AATATGCTTG	CCGGAAATAT	GGAAAATCAA	GCAACGGATG
401	CCGGGGAATC	GTCTCAGCCG	GCAAACCAAC	CGGATATGGC	AAATGCGGCG
451	GACGGAATGC	AGGGGGACGA	TCCGTCGGCA	GGCGGGCAAA	ATGCCGGCAA
501	TACGGCTGCC	CAAGGTGCAA	ATCAAGCCCG	AAACAATCAA	GCCGCCGGTT
551	CTTCAGATCC	CATCCCCGCG	TCAAACCTTG	CACCTGCGAA	TGGCGGTAGC
601	AATTTTGGAA	GGGTTGATT	GGCTAATGGC	GTTTTGATTG	ACGGGCCGTC
651	GCAAAATATA	ACGTTGACCC	ACTGTAAAGG	CGATTCTTGT	AGTGGCAATA
701	ATTTCTTGGA	TGAAGAAGTA	CAGCTAAAT	CAGAATTGTA	AAAATTAAGT
751	GATGCAGACA	AAATAAGTAA	TTACAAGAAA	GATGGGAAGA	ATGATAAATT
801	TGTCGGTTTG	GTTGCCGATA	GTGTGCAGAT	GAAGGGAATC	AATCAATATA
851	TTATCTTTTA	TAAACCTAAA	CCCCTTCAT	TTGCGCGATT	TAGGCGTTCT
901	GCACGGTCGA	GGCGGTGCGT	TCCGGCCGAG	ATGCCGCTGA	TTCCCGTCAA
951	TCAGGCGGAT	ACGCTGATTG	TCGATGGGGA	AGCGGTCAGC	CTGACGGGGC
1001	ATTCGGGCAA	TATCTTCGCG	CCCGAAGGGA	ATTACCGGTA	TCTGACTTAC
1051	GGGGCGGAAA	AATTGCCCGG	CGGATCGTAT	GCCCTTCGTG	TTCAAGCCGA
1101	ACCGGCAAAA	GGCGAAATGC	TTGCGGGCGC	GGCCGTGTAC	AACGGCGAAG
1151	TACTGCATTT	CCATACGGAA	AACGGCCGTC	CGTACCCGAC	CAGGGGCAGG
1201	TTTGCCGCAA	AAGTCGATT	CGGCAGCAAA	TCTGTGGACG	GCATTATCGA
1251	CAGCGGCGAT	GATTTCGATA	TGGGTACGCA	AAAATTCAAA	GCCGCCATCG
1301	ATGGAAACGG	CTTTAAGGGG	ACTTGACCGG	AAAATGGCAG	CGGGGATGTT
1351	TCCGGAAAGT	TTTACGGCCC	GGCCGGCGAG	GAAGTGGCGG	GAAAATACAG
1401	CTATCGCCCG	ACAGATGCGG	AAAAGGGCGG	ATTGCGCGTG	TTTGCCGGCA
1451	AAAAAGAGCA	GGATTGA			

This corresponds to the amino acid sequence <SEQ ID 69; ORF 287>:

m287.pep

1	MFKRSVIAMA	CIFALSACGG	GGGGSFDPVKS	ADTLSPKPAAP	VVSEKETEAK
51	EDAPQAGSQG	QGAPSAQGSQ	DMAAVSEENT	GNGGAVTADN	PKNEDEVAQN
101	DMPQNAAGTD	SSTPNHTPDP	NMLAGNMENQ	ATDAGESSQP	ANQPDMAANA
151	DGMQGGDDPSA	GGQNAAGNTAA	QGANQAGNNQ	AAGSSDFIPA	SNPAPANGGS
201	NFGRVVLANG	VLIDGPSQNI	TLTHCKGDSC	SGNNFLDEEV	QLKSEFEKLS
251	DADKISNYKK	DGKNDKFVGL	VADSVQMKGI	NQYIIFYKPK	PTSFAFRFRS
301	ARSRRSLPAE	MPLIPVNOAD	TLIVDGEAVS	LTGHSNIFA	PEGNYRYLTY

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351 GAEKLPGGSY ALRVQGEPAK GEMLAGAAVY NGEVLHFHTE NGRPYPTRGR
 401 FAAKVDGFSK SVDGIIDSGD DLHMGTKQFK AAIDGNGFKG TWTENGSGDV
 451 SGKFGYPAGE EVAGKYSYRP TDAEKGGFGV FAGKKEQD*

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 70>:

g287.seq
 1 atgtttaaac gcagtgtgat tgcaatggct tgtatttttc ccctttcagc
 51 ctgtgggggc ggcggtggcg gatcgcccga tgtcaagtcg gcggacacgc
 101 cgtcaaaacc ggcgcgcccc gttgttgctg aaaaatgccg ggaaggggtg
 151 ctgccgaaag aaaagaaaaga tgaggaggca gcgggcggtg cgccgcaagc
 201 cgatacgagc gacgcaaccg ccggagaagg cagccaagat atggcggcag
 251 tttcggcaga aaatacagcg aatggcggtg cggcaacaac ggacaacccc
 301 aaaaatgaag acgcgggggc gcaaaatgat atgcccgaat atgcccgcga
 351 atccgcgaat caaacaggga acaaccaacc cgccggttct tcagattccg
 401 ccccgcgctc aaaccctgcc cctgcgaatg gcggtagcga ttttggagg
 451 acgaacgtgg gcaattctgt tgtgattgac ggaccgtcgc aaaaataaac
 501 gttgaccac tgtaaaaggc attcttgtaa tggtgataat ttattggatg
 551 aagaagcacc gtcaaaatca gaatttgaaa aattaagtga tgaagaaaaa
 601 attaaagcga ataaaaaaga cgagcaacgg gagaattttg tcggtttggt
 651 tgctgacagg gtaaaaaagg atggaactaa caaatatata atcttctata
 701 cggacaaacc acctactcgt tctgcacggt cgaggaggtc gcttccggcc
 751 gagattccgc tgattcccg caatcaggcc gatacgctga ttgtggatgg
 801 ggaagcggtc agcctgacgg ggcattcccg caatatcttc gcgccgaag
 851 ggaattaccg gtatctgact tacggggcgg aaaaattgcc cgccgcatcg
 901 tatgccctcc gtgtgcaagg cgaaccggca aaaggcgaaa tgcttgttgg
 951 cacggcggcg tacaacggcg aagtgtgca tttccatatg gaaaacggcc
 1001 gtccgtaccc gtccggaggc aggtttgccg caaaagtcga tttcggcagc
 1051 aaatctgtgg acggcattat cgacagcggc gatgatttgc atatgggtac
 1101 gcaaaaattc aaagccgcca tcgatggaaa cggctttaag gggacttggg
 1151 cggaaaatgg cggcggggat gtttccggaa ggttttacgg cccggccggc
 1201 gaggaagtgg cgggaaaata cagctatcgc ccgacagat ctgaaaaggg
 1251 cggattcggc gtgtttgccg gcaaaaaaga tcgggattga

This corresponds to the amino acid sequence <SEQ ID 71; ORF 287.ng>:

g287.pep
 1 MFKRSVIAMA CIFPLSACGG GGGGSPDVKS ADTPSKPAAP VVAENAGEGV
 51 LPKEKKDEEA AGGAPQADTQ DATAGEGSD MAAVSAENTG NGGAATTDNP
 101 KNEDAGAQN MPQNAEASAN QTGNNQFAGS SDSAPASNPA PANGGSDFGR
 151 TNVGNVVID GPSQNTLTH CKGDSCNGDN LDDEEAPSKS EFEKLSDEEK
 201 IKRYKKDEQR ENFVGLVADR VKKDGTNKYI IFYTDKPTR SARSRRLPA
 251 EIPLIPVNA DTLIVDGEAV SLTGHSGNIF APEGNYRYLT YGAEKLPGGS
 301 YALRVQGEPA KGEMLVGTAV YNGEVLHFHM ENGRPYPSGG RFAAKVDGFS
 351 KSVDGIIDSG DDLHMGTKQF KAAIDGNGFK GTWTENGSGD VSGRFGYPAG
 401 EEVAGKYSYR PTDAEKGGFG VFAGKKDRD*

m287/g287 ORFs 287 and 287.ng showed a 70.1% identity in 499 aa overlap

	10	20	30	40	49
m287.pep	MFKRSVIAMACIFALSACGGGGGGSPDVKSADTLSPKPAAPVVSE-----	KETEAK			
g287	MFKRSVIAMACIFPLSACGGGGGGSPDVKSADTPSKPAAPVVAENAGEGVLPKEKKDEEA				
	10	20	30	40	50
	50	60	70	80	90
m287.pep	KEDAPQAGSQGQGPSAQGSQDMAAVSEENTGNGGAVTADNPKNEDEVAQNMPQNAAGT				
g287	AGGAPQADTQD--ATAGEGSDMAAVSAENTGNGGAATTDNPKNEDAGAQNMPQNA--				
	70	80	90	100	110

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m287.pep	110	120	130	140	150	160	169
	DSSTPNHTPDNMLAGNMENQATDAGESSQFANQPDMANAADGMQGDPSAGGQNAGNTA						
g287	-----						
m287.pep	170	180	190	200	210	220	229
	AQGANQAGNNQAAGSSDPIPASNPAPANGGSNFGVRVDLANGVLIDGPSQNTITLTHCKGDS						
g287	-ESANQTGNNQPAGSSDSAPASNPAPANGGSDFGRTNVGNVVIDGPSQNTITLTHCKGDS						
	120	130	140	150	160	170	
m287.pep	230	240	250	260	270	280	289
	CSGNNFLDEEVQLKSEFEKLSADAKISNYKKDGKNDKFVGLVADSVQMKGINQYIIFYKP						
g287	CNGDNLLDEEAPSKSEFEKLSDEEKIKRYKKDEQRENFVGLVADRVKKDGTNKYIIFYTD						
	180	190	200	210	220	230	
m287.pep	290	300	310	320	330	340	349
	KPTSFARFRRSARSRRSLPAEMPLIPVNQADTLIVDGEAVSLTGHSGNIFAPEGNYRYLT						
g287	KPPT-----RSARSRRSLPAEIPVNPQADTLIVDGEAVSLTGHSGNIFAPEGNYRYLT						
	240	250	260	270	280	290	
m287.pep	350	360	370	380	390	400	409
	YGAEKLPGGSYALRVQGEPAKGEMLAGAAVYNGEVLHFHTENGRPYPTRGRFAAKVDFGS						
g287	YGAEKLPGGSYALRVQGEPAKGEMLVGTAVYNGEVLHFHMENGRPYPSGGRFAAKVDFGS						
	300	310	320	330	340	350	
m287.pep	410	420	430	440	450	460	469
	KSVDGIIDSGDDLHMGTKQFKAAIDGNGFKGTWTENGSGDVSCKFYGPAGEEVAGKYSYR						
g287	KSVDGIIDSGDDLHMGTKQFKAAIDGNGFKGTWTENGSGDVSGRFYGPAGEEVAGKYSYR						
	360	370	380	390	400	410	
m287.pep	470	480	489				
	PTDAEKGFGVFAGKKEQDX						
g287	PTDAEKGFGVFAGKKDRDX						
	420	430					

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 72>:

```

a287.seq
1   ATGTTTAAAC GCAGTGTGAT TGCAATGGCT TGTATTGTTG CCCTTTCAGC
51  CTGTGGGGGC GGCAGTGGCG GATCGCCCGA TGTTAAGTCG GCGGACACGC
101 TGTCAAAACC TGCCGCCCTT GTTGTACTG AAGATGTCGG GGAAGAGGTG
151 CTGCCGAAAG AAAAGAAAGA TGAGGAGGCG GTGAGTGGTG CGCCGCAAGC
201 CGATACGCAG GACGCAACCG CCGGAAAAGG CGGTCAAGAT ATGGCGGCAG
251 TTTTCGGCAGA AAATACAGGC AATGGCGGTG CGGCAACAAC GGATAATCCC
301 GAAAATAAAG ACGAGGGACC GCAAAATGAT ATGCCGCAAA ATGCCGCCGA
351 TACAGATAGT TCGACACCGA ATCACACCCC TGCACCGAAT ATGCCAACCA
401 GAGATATGGG AAACCAAGCA CCGGATGCCG GGAATCGGC ACAACCGGCA
451 AACCAACCGG ATATGGCAAA TCGCGCGGAC GGAATGCAGG GGGACGATCC
501 GTCGCGAGGG GAAAATGCCG GCAATACGGC AGATCAAGCT GCAAAATCAAG
551 CTGAAAACAA TCAAGTCGGC GGCTCTCAAA ATCCTGCCTC TTCAACCAAT
601 CCTAACGCCA CGAATGGCGG CAGCGATTTT GGAAGGATAA ATGTAGCTAA
651 TGGCATCAAG CTTGACAGCG GTTCGGAAAA TGTACGTTG ACACATTGTA
701 AAGACAAAGT ATGCGATAGA GATTCTTAG ATGAAGAAGC ACCACCAAAA
751 TCAGAATTTG AAAAATTAAG TGATGAAGAA AAAATTAATA AATATAAAAA
801 AGACGAGCAA CGAGAGAATT TTGTCGGTTT GGTGCTGAC AGGGTAGAAA

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851 AGAATGGAAC TAACAAATAT GTCATCATTT ATAAAGACAA GTCCGCTTCA
901 TCTTCATCTG CGCGATTGAG GCGTTCTGCA CGGTCGAGGC GGTCGCTTCC
951 GGCCGAGATG CCGCTGATTC CCGTCAATCA GCGGATACG CTGATTGTGCG
1001 ATGGGGAAGC GGTGAGCCTG ACGGGGCATT CCGGCAATAT CTTCGCGCCC
1051 GAAGGGAATT ACCCGTATCT GACTTACGGG GCGGAAAAAT TGTCCGGCGG
1101 ATCGTATGCC CTCAGTGTGC AAGGCGAACC GGCAAAAGGC GAAATGCTTG
1151 CGGGCACGGC CGTGTACAAC GCGAAGTGC TGCATTTCCA TATGAAAAAC
1201 GGCCGTCCGT CCCCCTCCGG AGGCAGGTTT GCCGCAAAAG TCGATTTCCG
1251 CAGCAAATCT GTGGACGGCA TTATCGACAG CGGCGATGAT TTGCATATGG
1301 GTACGCAAAA ATTCAAAGCC GTTATCGATG GAAACGGCTT TAAGGGGACT
1351 TGGACGAAAA ATGGCGGCGG GGATGTTTCC GGAAGGTTT ACGGCCCGGC
1401 CGGCGAAGAA GTGGCGGGAA AATACAGCTA TCGCCCGACA GATGCGGAAA
1451 AGGCGGATT CCGCGTGTTC GCCGCAAAA AAGAGCAGGA TTGA

```

This corresponds to the amino acid sequence <SEQ ID 73; ORF 287.a>:

```

a287.pep
1 MFKRSVIAMA CIVALSAACGG GGGGSPDVKS ADTLSKPAAP VVTEDVGEEV
51 LPKEKKDEEA VSGAPQADTQ DATAGKGGQD MAAVSAENTG NGGAATTDNP
101 ENKDEGPOND MPQNAADTDS STPNHTPAPN MPTRDMGNQA PDAGESAQPA
151 NQPDMANAAD GMQGGDPSAG ENAGNTADQA ANQAENNQVG GSNPASSTN
201 PNATNGGSDF GRINVANGIK LDGSENVTL THCKDKVCDR DFLDEEAPPK
251 SEFEKLSDEE KINKYKKDEQ RENFVGLVAD RVEKNGTNKY VIIYKDKSAS
301 SSSARFRRSA RSRRLPAEM PLIPVNOADT LIVDGEAVSL TGHSGNIFAP
351 EGNRYRLTYG AEKLSGGSYA LSVQGEPAKG EMLAGTAVYN GEVLHFHMEN
401 GRPSPSGGRF AAKVDFGSKS VDGIIIDSGD LHMGTQKFKA VIDGNGFKGT
451 WTENGGDVVS GRFYGPAGEE VAGKYSYRPT DAEGGFGVF AGKKEQD*

m287/a287 ORFs 287 and 287.a showed a 77.2% identity in 501 aa overlap

10 20 30 40 49
m287.pep MFKRSVIAMACIFALSACGGGGGGSPDVKSADTLSKPAAPVVSE-----KETEA
||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
a287 MFKRSVIAMACIVALSAACGGGGGGSPDVKSADTLSKPAAPVVTEDVGEEVLPKEKKDEEA
10 20 30 40 50 60

50 60 70 80 90 100 109
m287.pep KEDAPQAGSQGQAPSAQGSQDMAAVSEENTGNGGAVTADNPKNEDEVAQNMDMPQNAAGT
||| :| |:::||||| |||||:|:|:|:|:| ||||| ||||| |||||
a287 VSGAPQADTQ--DATAGKGGQDMAAVSAENTGNGGAATTDNPENKDEGPONDMPQNAADT
70 80 90 100 110

110 120 130 140 150 160 169
m287.pep DSSTPNHTPDFNMLAGNMENQATDAGESSQPANQPDMANAADGMQGGDPSAGGQNAGNTA
||||| ||| :| ||| |||||:||||| |||||:||||| |||||:||||| |||||
a287 DSSTPNHTPAPNMPTRDMGNQAPDAGESAQPANQPDMANAADGMQGGDPSAG-ENAGNTA
120 130 140 150 160 170

170 180 190 200 210 220 229
m287.pep AQGANQAGNNQAAGSSDPIPASNPAPANGGSNFRVLDLANGVLIDGPSQNTLTHCKGDS
|:|||| |:|:|:|:| |:| |:|:|:|:|:|:| |:| |:|:|:|:|
a287 DQAAQAENNQVGGSONPASSTNPATNGGSDFRINVANGIKLDGSENVTLTHCKDKV
180 190 200 210 220 230

230 240 250 260 270 280 289
m287.pep CSGNNFLDEEVQLKSEFEKLSADAKISNYKKDGKNDKFVGLVADSVQMKGINQYIIFYKP
|: |:|:|:|:| ||||| |:|:|:|:| |:|:|:|:| |:| |:|:|:|:|
a287 CD-RDFLDEEAPPKSEFEKLSDEEKINKYKKDEQRENFVGLVADRVKNGTNKYVIIYKD
240 250 260 270 280 290

290 300 310 320 330 340
m287.pep KP--TSFARFRRSARSRRSLPAEMPLIPVNOADTLIVDGEAVSLTGHSGNIFAPEGNRY
| :| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||

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a287      KSASSSSARFRRSARSRRSLPAEMPLIPVNQADTLIVDGEAVSLTGHSGNIFAPEGNRYR
           300      310      320      330      340      350

m287.pep  350      360      370      380      390      400
           LTYGAEKLPGGSYALRVQGEPAKGEMLAGAAVYNGEVLHFHTENGRPYPTRGRFAAKVDF
           ||||| ||||| ||||| |||||:||||| ||||| ||||| ||||| |||||
a287      LTYGAEKLSGGSYALSVQGEPAKGEMLAGTAVYNGEVLHFHMENGRPSPSGGRFAAKVDF
           360      370      380      390      400      410

m287.pep  410      420      430      440      450      460
           GSKSVDSGIIDSGDDLHMGTKQFKAAIDGNGFKGTWTENGSGDVSGKFYGPAGEEVAGKYS
           ||||| ||||| ||||| |||||:||||| ||||| ||||| ||||| |||||
a287      GSKSVDSGIIDSGDDLHMGTKQFKAAIDGNGFKGTWTENGSGDVSGRFYGPAGEEVAGKYS
           420      430      440      450      460      470

m287.pep  470      480      489
           YRPTDAEKGFGVGFAGKKEQDX
           ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
a287      YRPTDAEKGFGVGFAGKKEQDX
           480      490

```

406

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 74>:

```

m406.seq
1  ATGCAAGCAC GGCTGCTGAT ACCTATTCTT TTTTCAGTTT TTATTTTATC
51  CGCCTGCGGG ACACTGACAG GTATTCCATC GCATGGCGGA GGTAAACGCT
101 TTGCGGTCGA ACAAGAACTT GTGGCCGCTT CTGCCAGAGC TGCCGTTAAA
151 GACATGGATT TACAGGCATT ACACGGACGA AAAGTTGCAT TGTACATTGC
201 CACTATGGGC GACCAAGGTT CAGGCAGTTT GACAGGGGGT CGCTACTCCA
251 TTGATGCACT GATTTCGTGGC GAATACATAA ACAGCCCTGC CGTCCGTACC
301 GATTACACCT ATCCACGTTA CGAAACCACC GCTGAAACAA CATCAGGCGG
351 TTTGACAGGT TTAACCACTT CTTTATCTAC ACTTAATGCC CCTGCACTCT
401 CTCGCACCCA ATCAGACGGT AGCGGAAGTA AAAGCAGTCT GGGCTTAAAT
451 ATTGGCGGGA TGGGGGATTA TCGAAATGAA ACCTTGACGA CTAACCCGCG
501 CGACACTGCC TTTCTTTCCC ACTTGGTACA GACCGTATTT TTCCTGCGCG
551 GCATAGACGT TGTTTCTCCT GCCAATGCCG ATACAGATGT GTTTATTAAC
601 ATCGACGTAT TCGGAACGAT ACGCAACAGA ACCGAAATGC ACCTATACAA
651 TGCCGAAACA CTGAAAGCCC AAACAAACT GGAATATTTT GCAGTAGACA
701 GAACCAATAA AAAATTGCTC ATCAAACCAA AAACCAATGC GTTTGAAGCT
751 GCCTATAAAG AAAATTACGC ATTGTGGATG GGGCCGTATA AAGTAAGCAA
801 AGGAATTAAA CCGACGGAAG GATTAATGGT CGATTTCTCC GATATCCGAC
851 CATACGGCAA TCATACGGGT AACTCCGCCC CATCCGTAGA GGCTGATAAC
901 AGTCATGAGG GGTATGGATA CAGCGATGAA GTAGTGCAC AACATAGACA
951 AGGACAACCT TGA

```

This corresponds to the amino acid sequence <SEQ ID 75; ORF 406>:

```

m406.pep
1  MQARLLIPIL FSVFILSACG TLTGIPSHGG GKRFVEQEL VAASARAAVK
51  DMDLQALHGR KVALYIATMG DQSGSLTGG RYSIDALIRG EYINSPAVRT
101 DYTPRYETT AETTSGLTG LTTSLSTLNA PALSRQSDG SGSKSSLGLN
151 IGGMDYRNE TLTNPRDTA FLSHLVQTVF FLRGIDVVSP ANADTDVFIN
201 IDVFGTIRNR TEMHLYNAET LKAQTKLEYF AVDRTNKLL IKPKTNAFEA
251 AYKENYALWM GPYKVSIGIK PTEGLMVDfs DIRPYGNHTG NSAPSVEADN
301 SHEGYGYSDE VVRQHRQGQP *

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The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 76>:

g406.seq

```

1   ATGCGGGCAC GGCTGCTGAT ACCTATTCTT TTTTCAGTTT TTATTTTATC
51  CGCCTGCGGG AACTGACAG GTATTCCATC GCATGGCGGA GGCAAACGCT
101 TCGCGGTCGA ACAAGAACTT GTGGCCGCTT CTGCCAGAGC TGCCGTTAAA
151 GACATGGATT TACAGGCATT ACACGGACGA AAAGTTGCAT TGTACATTGC
201 AACTATGGGC GACCAAGGTT CAGGCAGTTT GACAGGGGGT CGCTACTCCA
251 TTGATGCACT GATTGCGGGC GAATACATAA ACAGCCCTGC CGTCCGCACC
301 GATTACACCT ATCCGCGTTA CGAAACCACC GCTGAAACAA CATCAGGCGG
351 TTTGACGGGT TTAACCACTT CTTTATCTAC ACTTAATGCC CCTGCACTCT
401 CGCGCACCCA ATCAGACGGT AGCGGAAGTA GGAGCAGTCT GGGCTTAAAT
451 ATTGCGGGGA TGGGGGATTA TCGAAATGAA ACCTTGACGA CCAACCCGCG
501 CGACACTGCC TTTCTTTCCC ACTTGGTGCA GACCGTATTT TTCCTGCGCG
551 GCATAGACGT TGTTCCTCCT GCCAATGCCG ATACAGATGT GTTTATTAAAC
601 ATCGACGTAT TCGGAACGAT ACGCAACAGA ACCGAAATGC ACCTATACAA
651 TGCCGAAACA CTGAAAGCCC AAACAAAACCT GGAATATTTT GCAGTAGACA
701 GAACCAATAA AAAATTGCTC ATCAAACCCA AAACCAATGC GTTTGAAGCT
751 GCCTATAAAG AAAATTACGC ATTGTGGATG GGGCCGTATA AAGTAAGCAA
801 AGGAATCAAA CCGACGGAAG GATTGATGGT CGATTTCTCC GATATCCAAC
851 CATACGGCAA TCATACGGGT AACTCCGCCC CATCCGTAGA GGCTGATAAC
901 AGTCATGAGG GGTATGGATA CAGCGATGAA GCAGTGCAC AACATAGACA
951 AGGGCAACCT TGA

```

This corresponds to the amino acid sequence <SEQ ID 77; ORF 406.ng>:

g406.pep

```

1   MRARLLIPIL FSVFILSACG TLTGIPSHGG GKRFVEQEL VAASARAANK
51  DMDLQALHGR KVALYIATMG DQSGSLTGG RYSIDALIRG EYINSPAVRT
101 DYTPRYETT AETTSGLTG LTSLSTLNA PALSRQSDG SGRSSLGLN
151 IGGMGDYRNE TLTTNPRDTA FLSHLVQTVF FLRGIDVVSF ANADTDVFIN
201 IDVFGTIRNR TEMHLYNAET LKAQTKLEYF AVDRTNKLL IKPKTNAFEA
251 AYKENYALWM GPYKVSQGIK PTEGLMVDPS DIQPYGNHTG NSAPSVEADN
301 SHEGYGYSDE AVRQHRQGP *

```

ORF 406.ng shows 98.8% identity over a 320 aa overlap with a predicted ORF (ORF406.a) from *N. gonorrhoeae*:

g406/m406

	10	20	30	40	50	60
g406.pep	MRARLLIPILFSVFILSACGLTGIPSHGGGKRFVEQELVAASARAANKDMDLQALHGR					
m406	MQARLLIPILFSVFILSACGLTGIPSHGGGKRFVEQELVAASARAANKDMDLQALHGR					
	10	20	30	40	50	60
g406.pep	KVALYIATMGDQSGSLTGGGRYSIDALIRGEYINSPAVRTDYTPRYETTAETTSGLTG					
m406	KVALYIATMGDQSGSLTGGGRYSIDALIRGEYINSPAVRTDYTPRYETTAETTSGLTG					
	70	80	90	100	110	120
g406.pep	KVALYIATMGDQSGSLTGGGRYSIDALIRGEYINSPAVRTDYTPRYETTAETTSGLTG					
m406	KVALYIATMGDQSGSLTGGGRYSIDALIRGEYINSPAVRTDYTPRYETTAETTSGLTG					
	70	80	90	100	110	120
g406.pep	LTSLSTLNAPALSRQSDGSGSSLGLNIGMGDYRNETLTNPRDTAFLSHLVQTVF					
m406	LTSLSTLNAPALSRQSDGSGSKSSLGLNIGMGDYRNETLTNPRDTAFLSHLVQTVF					
	130	140	150	160	170	180
g406.pep	LTSLSTLNAPALSRQSDGSGSKSSLGLNIGMGDYRNETLTNPRDTAFLSHLVQTVF					
m406	LTSLSTLNAPALSRQSDGSGSKSSLGLNIGMGDYRNETLTNPRDTAFLSHLVQTVF					
	130	140	150	160	170	180
	190	200	210	220	230	240

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g406.pep  FLRGIDVVSPANADTDVFINIDVFGTIRNRTEMHLYNAETLKAQTKLEYFAVDRTNKKLL
|||||
m406      FLRGIDVVSPANADTDVFINIDVFGTIRNRTEMHLYNAETLKAQTKLEYFAVDRTNKKLL
              190      200      210      220      230      240

              250      260      270      280      290      300
g406.pep  IKPKTNAFEAAAYKENYALWMGPYKVSIGIKPTEGLMVDFSIDIQPYGNHTGNSAPSVEADN
|||||
m406      IKPKTNAFEAAAYKENYALWMGPYKVSIGIKPTEGLMVDFSIDIQPYGNHTGNSAPSVEADN
              250      260      270      280      290      300

              310      320
g406.pep  SHEGYGYSDEAVRQHRQGQPX
|||||
m406      SHEGYGYSDEVVRQHRQGQPX
              310      320

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 78>:

```

a406.seq
1  ATGCAAGCAC GGCTGCTGAT ACCTATTCTT TTTTCAGTTT TTATTTTATC
51  CGCCTGCGGG ACACTGACAG GTATTCCATC GCATGGCGGA GGTAACGCT
101 TCGCGGTCGA ACAAGAACTT GTGGCCGCTT CTGCCAGAGC TGCCGTTAAA
151 GACATGGATT TACAGGCATT ACACGGACGA AAAGTTGCAT TGTACATTGC
201 AACTATGGGC GACCAAGGTT CAGGCAGTTT GACAGGGGGT CGTACTCCA
251 TTGATGCACT GATTTCGTGGC GAATACATAA ACAGCCCTGC CGTCCGTACC
301 GATTACACCT ATCCACGTTA CGAAACCACC GCTGAAACAA CATCAGGCGG
351 TTTGACAGGT TTAACCACTT CTTTATCTAC ACTTAATGCC CCTGCACTCT
401 CGCGCACCCA ATCAGACGGT AGCGGAAGTA AAAGCAGTCT GGGCTTAAAT
451 ATTGGCGGGA TGGGGGATTA TCGAAATGAA ACCTTGACGA CTAACCCGCG
501 CGACACTGCC TTTCTTTCCC ACTTGGTACA GACCGTATTT TTCCTGCGCG
551 GCATAGACGT TGTTCCTCCT GCCAATGCCG ATACGGATGT GTTTATTAAC
601 ATCGACGTAT TCGGAACGAT ACGCAACAGA ACCGAAATGC ACCTATACAA
651 TGCCGAAACA CTGAAAGCCC AAACAAACT GGAATATTTC GCAGTAGACA
701 GAACCAATAA AAAATTGCTC ATCAAACCAA AAACCAATGC GTTTGAAGCT
751 GCCTATAAAG AAAATTACGC ATTGTGGATG GGACCGTATA AAGTAAGCAA
801 AGGAATTAAA CCGACAGAAG GATTAATGGT CGATTCTCC GATATCCAAC
851 CATACGGCAA TCATATGGGT AACTCTGCCC CATCCGTAGA GGCTGATAAC
901 AGTCATGAGG GGTATGGATA CAGCGATGAA GCAGTGCGAC GACATAGACA
951 AGGGCAACCT TGA

```

This corresponds to the amino acid sequence <SEQ ID 79; ORF 406.a>:

```

a406.pep
1  MQARLLIPIL FSVFILSACG TLTGIPSHGG GKRFAVEQEL VAASARAANK
51  DMDLQALHGR KVALYIATMG DQSGSLTGG RYSIDALIRG EYINSPAVRT
101 DYTYPRYETT AETTSGLTG LTSLSTLNA PALSRQSDG SGSKSSLGLN
151 IGGMGDYRNE TLTTNPRDTA FLSHLVQTVF FLRGIDVVSP ANADTDVFIN
201 IDVFGTIRNR TEMHLYNAET LKAQTKLEYF AVDRTNKKLL IKPKTNAFEA
251 AYKENYALWM GPYKVSIGIK PTEGLMVDFS IDIOPYGNHM NSAPSVEADN
301 SHEGYGYSDE AVRRHRQGQP *

m406/a406  ORFs 406 and 406.a showed a 98.8% identity in 320 aa overlap

              10      20      30      40      50      60
m406.pep  MQARLLIPILFSVFILSACGTLTGIPSHGGGKRFAVEQELVAASARAANKDMDLQALHGR
|||||
a406      MQARLLIPILFSVFILSACGTLTGIPSHGGGKRFAVEQELVAASARAANKDMDLQALHGR
              10      20      30      40      50      60

              70      80      90      100     110     120
m406.pep  KVALYIATMGDQSGSLTGGRYSIDALIRGEYINSPAVRTDYTYPRYETTAETTSGLTG
|||||

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a406      KVALYIATMGDQSGSLTGGRYSIDALIRGEYINSPAVRTDYTPRYETTAETSSGGLTG
           70      80      90      100     110     120

           130     140     150     160     170     180
m406.pep  LTTSLSLTLNAPALSRTQSDGSGSKSSLGLNIGGMGDYRNETLTNPNRDTAFLSHLVQTVF
           |||
a406      LTTSLSLTLNAPALSRTQSDGSGSKSSLGLNIGGMGDYRNETLTNPNRDTAFLSHLVQTVF
           130     140     150     160     170     180

           190     200     210     220     230     240
m406.pep  FLRGIDVVS PANADTDVFINIDVFGTIRNRTEMHLYNAETLKAQTKLEYFAVDRTNKKLL
           |||
a406      FLRGIDVVS PANADTDVFINIDVFGTIRNRTEMHLYNAETLKAQTKLEYFAVDRTNKKLL
           190     200     210     220     230     240

           250     260     270     280     290     300
m406.pep  IKPKTNAFEAAAYKENYALWMGPYKVS KGIKPTEGLMVD FSDIRPYGNH TGNAPSVEADN
           |||
a406      IKPKTNAFEAAAYKENYALWMGPYKVS KGIKPTEGLMVD FSDIQPYGNH MGNAPSVEADN
           250     260     270     280     290     300

           310     320
m406.pep  SHEGYGYSDEVVRQHRQGQPX
           |||
a406      SHEGYGYSDEAVRRHRQGQPX
           310     320

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 80>:

```

m726.seq
1   ATGACCATCT ATTTCAAAAA CGGCTTTTAC GACGACACAT TGGGCGGCAT
51  CCCCGAAGGC GCGGTGCGCG TCCGCGCCGA AGAATACGCC GCCCTTTTGG
101 CAGGACAGGC GCAGGGCGGG CAGATTGCCG CAGATTCCGA CGGCCGCCCC
151 GTTTTAACCC CGCCGCGCCC GTCCGATTAC CACGAATGGG ACGGCAAAAA
201 ATGGAAAATC AGCAAAGCCG CCGCCGCCGC CCGTTTCGCC AAACAAAAAA
251 CCGCCTTGGC ATTCCGCCTC GCGGAAAAGG CGGACGAACT CAAAAACAGC
301 CTCTTGGCGG GCTATCCCCA AGTGGAAATC GACAGCTTTT ACAGGCAGGA
351 AAAAGAAGCC CTCGCGCGGC AGGCGGACAA CAACGCCCCG ACCCCGATGC
401 TGGCGCAAAT CGCCGCCGCA AGGGGCGTGG AATTGGACGT TTTGATTGAA
451 AAAGTTATCG AAAAATCCGC CCGCCTGGCT GTTGCCGCCG GCGCGATTAT
501 CGGAAAGCGT CAGCAGCTCG AAGACAAATT GAACACCATC GAAACCGCGC
551 CCGGATTGGA CGCGCTGGAA AAGGAAATCG AAGATGGAC GCTAAACATC
601 GGCTGA

```

This corresponds to the amino acid sequence <SEQ ID 81; ORF 726>:

```

m726.pep
1   MTIYFKNGFY DDTLGGIPEG AVAVRAEEYA ALLAGQAQGG QIAADSDGRP
51  VLTPPRPSDY HEWDGKKWKI SKAAAAARFA KQKTALAFRL AEKADELKNS
101 LLAGYPQVEI DSFYRQEKEA LARQADNNAP TPMLAQIAAA RGVELDLVIE
151 KVIKESARLA VAAGAIIGKR QQLEDKLNIT ETAPGLDALE KEIEEWTLNI
201 G*

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 82>:

```

m907-2.seq
1   ATGAGAAAAC CGACCGATAC CCTACCCGTT AATCTGCAAC GCCGCCGCCT
51  GTTGTGTGCC GCCGGTGC GTTGTCTCAG TCCTCTGGCG CAGCCGGCG
101 CGCAACGTGA GGAAACGCTT GCCGACGATG TGGCTTCCGT GATGAGGAGT

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151 TCTGTCGGCA GCGTCAATCC GCCGAGGCTG GTGTTTGACA ATCCGAAAGA
201 GGGCGAGCGT TGGTTGTCTG CCATGTCGGC ACGTTTGGA AGGTTCGTCC
251 CCGAGGAGGA GGAGCGGCGC AGGCTGCTGG TCAATATCCA GTACGAAAGC
301 AGCCGGGCGG GTTGGATAC GCAGATTGTG TTGGGGCTGA TTGAGGTGGA
351 AAGCGCGTTC CGCCAGTATG CAATCAGCGG TGTCGGCGCG CGCGGCCTGA
401 TGCAGGTATG GCCGTTTGG AAAAATAACA TCGGCAAAAC GCGGCACAAAC
451 CTGTTTCGACA TCCGCACCAA CCTGCGTTAC GGCTGTACCA TCCTGCGCCA
501 TTACCGGAAT CTTGAAAAAG GCAACATCGT CCGCGCGCTT GCCCGCTTTA
551 ACGGCAGCTT GGGCAGCAAT AAATATCCGA ACGCCGTTT GGGCGCGTGG
601 CGCAACCGCT GGCAGTGGCG TTGA

```

This corresponds to the amino acid sequence <SEQ ID 83; ORF 907-2>:

m907-2.pep

```

1 MRKPTDTLPV NLQRRRLCA AGALLLSPLA HAGAQREEL ADDVASVMRS
51 SVGSVNPRL VFDNPKEGER WLSAMSARLA RFVPEEEERR RLLVNIQYES
101 SRAGLDTQIV LGLIEVESAF RQYAISGVGA RGLMQVMPFW KNYIGKPAHN
151 LFDIRTNLRY GCTILRHYRN LEKGNIVRAL ARFNGSLGSN KYPNAVLGAW
201 RNRWQWR*

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 84>:

m953.seq

```

1 ATGAAAAAAA TCATCTTCGC CGCACTCGCA GCCGCCGCCA TCAGTACTGC
51 CTCCGCCGCC ACCTACAAAG TGGACGAATA TCACGCCAAC GCCCGTTTCG
101 CCATCGACCA TTTCAACACC AGCACCACCG TCGGCGGTTT TTACGGTCTG
151 ACCGGTTCCG TCGAGTTTGA CCAAGCAAAA CGCGACGGTA AAATCGACAT
201 CACCATCCCC ATTGCCAACG TGCAAAGCGG TTCGCAACAC TTACCGGACC
251 ACCTGAAATC AGCCGACATC TTCGATGCCG CCCAATATCC GGACATCCGC
301 TTTGTTTCCA CCAAATTCAA CTTCAACGGC AAAAACTGG TTCCGTTGA
351 CGGCAACCTG ACCATGCACG GCAAAACCGC CCCCGTCAAA CTCAAAGCCG
401 AAAAATTCAA CTGCTACCAA AGCCCGATGG AGAAAACCGA AGTTGTGGC
451 GCGGACTTCA GCACCACCAT CGACCGCACC AAATGGGGCA TGGACTACCT
501 CGTTAACGTT GGTATGACCA AAAGCGTCCG CATCGACATC CAAATCGAGG
551 CAGCCAAACA ATAA

```

This corresponds to the amino acid sequence <SEQ ID 85; ORF 953>:

m953.pep

```

1 MKKIIFAALA AAAISTASAA TYKVDEYHAN ARFAIDHFNT STNVGGFYGL
51 TGSVEFDQAK RDGKIDITIP IANLOSQSQH FTDHLKSADI FDAQYPPDIR
101 FVSTKFNENG KKLVSVDGNL TMHGKTAPVK LKAEKFNCYQ SPMEKTEVCG
151 GDFSTTIDRT KWGMDYLVNV GMTKSVRIDI QIEAAKQ*

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 86>:

orf1-1.seq

```

1 ATGAAAACAA CCGACAAACG GACAACCGAA ACACACCGCA AAGCCCCGAA
51 AACCGGCCGC ATCGGCTTCT CGCCTGCTTA CTTAGCCATA TGCCTGTCTG
101 TCGGCATTCT TCCCCAAGCC TGGCGGGGAC ACACTTATTT CGGCATCAAC
151 TACCAATACT ATCGCGACTT TGCCGAAAT AAAGGCAAGT TTGCAGTCGG
201 GCGGAAAGAT ATTGAGGTTT ACAACAAAAA AGGGGAGTTG GTCGGCAAAT
251 CAATGACAAA AGCCCCGATG ATTGATTTTT CTGTGGTGTC GCGTAACGGC
301 GTGGCGGCAT TGGTGGGCGA TCAATATATT GTGAGCGTGG CACATAACGG
351 CCGCTATAAC AACGTTGATT TTGGTGGCGA AGGAAGAAAT CCCGATCAAC
401 ATCGTTTAC TTATAAAATT GTGAAACGGA ATAATTATAA AGCAGGGACT
451 AAAGGCCATC CTTATGGCGG CGATTATCAT ATGCCGCGTT TGCATAAATT
501 TGTACAGAT GCAGAACCTG TTGAAATGAC CAGTTATATG GATGGGCGGA

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551 AATATATCGA TCAAAATAAT TACCCTGACC GTGTTCGTAT TGGGGCAGGC
 601 AGGCAATATAT GGCATCTGA TGAAGATGAG CCCAATAACC GCGAAAGTTC
 651 ATATCATATAT GCAAGTGCGT ATTCTTGGCT CGTTGGTGCG AATACCTTTG
 701 CACAAAATGG ATCAGGTGGT GGCACAGTCA ACTTAGGTAG TGAAAAAATT
 751 AAACATAGCC CATATGGTTT TTTACCAACA GGAGGCTCAT TTGGCGACAG
 801 TGGCTCACCA ATGTTTATCT ATGATGCCCA AAAGCAAAAG TGGTTAATTA
 851 ATGGGGTATT GCAAACGGGC AACCCCTATA TAGGAAAAAG CAATGGCTTC
 901 CAGCTGGTTC GTAAAGATTG GTTCTATGAT GAAATCTTTG CTGGAGATAC
 951 CCATTTCAGTA TTCTACGAAC CACGTCAAAA TGGGAAATAC TCTTTTAACG
 1001 ACGATAATAA TGGCACAGGA AAAATCAATG CCAAACATGA ACACAATTCT
 1051 CTGCCTAATA GATTAAAAAC ACGAACCGTT CAATTGTTTA ATGTTTCTTT
 1101 ATCCGAGACA GCAAGAGAAC CTGTTTATCA TGCTGCAGGT GGTGTCAACA
 1151 GTTATCGACC CAGACTGAAT AATGGAGAAA ATATTTCCTT TATTGACGAA
 1201 GTAAAAGGCG AATTGATACT TACCAGCAAC ATCAATCAAG GTGCTGGAGG
 1251 ATTATATTTC CAAGGAGATT TTACGGTCTC GCCTGAAAAA AACGAACTT
 1301 GGCAGGCGC GGGCGTTCAT ATCAGTGAAG ACAGTACCGT TACTTGAAAA
 1351 GTAAACGGCG TGGCAACGGA CCGCTGTCC AAAATCGGCA AAGGCACGCT
 1401 GCACGTTCAA GCCAAAGGGG AAAACCAAGG CTCGATCAGC GTGGCGGACG
 1451 GTACAGTCAT TTTGGATCAG CAGGCAGACG ATAAAGGCAA AAAACAAGCC
 1501 TTTAGTGAAT TCGCTTGGT CAGCGGCAGG GGTACGGTGC AACTGAATGC
 1551 CGATAATCAG TTCAACCCCG ACAAACCTCTA TTTCCGGCTTT CGCGGCGGAC
 1601 GTTTGGATT TAAACGGGCAT TCGCTTTCGT TCCACCGTAT TCAAATACCC
 1651 GATGAAGGGG CGATGATTGT CAACCACAAAT CAAGACAAAG AATCCACCGT
 1701 TACCATTACA GGCAATAAAG ATATTGCTAC AACCGGCAAT AACACAGCT
 1751 TGGATAGCAA AAAAGAAATT GCCTACAACG GTTGGTTTGG CGAGAAAGAT
 1801 ACGACCAAAA CGAACGGGCG GCTCAACCTT GTTTACCAGC CCGCCGCGAG
 1851 AGACCGCACC CTGCTGCTTT CCGCGGGAAC AAATTAAAC GGCAACATCA
 1901 CGCAACAAA CGGCAACTG TTTTCAGCG GCAGACCAAC ACCGCACGCC
 1951 TACAATCAT TAAACGACCA TTGGTCGCAA AAAGAGGGCA TTCCTCGCGG
 2001 GGAAATCGT TGGGACAACG ACTGGATCAA CCGCACATTT AAAGCGGAAA
 2051 ACTTCCAAAT TAAAGGCGGA CAGGCGGTGG TTTCCCGCAA TGTTGCCAAA
 2101 GTGAAAGGCG ATTGGCATT GAGCAATCAC GCCCAAGCAG TTTTGGTGT
 2151 CGCACCGCAT CAAAGCCACA CAATCTGTAC ACGTTCGGAC TGGACGGGTC
 2201 TGACAAATTG TGTGAAAAAA ACCATTACCG ACGATAAAGT GATTGCTTCA
 2251 TTGACTAAGA CCGACATCAG CCGCAATGTC GATCTGCGG ATCAGCTCA
 2301 TTTAAATCTC ACAGGGCTTG CCACACTCAA CGGCAATCTT AGTGCAAAATG
 2351 GCGATACAG TTATACAGT AGCCACAACG CCACCAAAA CGGCAACCTT
 2401 AGCCTCGTGG GCAATGCCCA AGCAACATTT AATCAAGCCA CATTAAACGG
 2451 CAACACATCG GCTTCGGGCA ATGCTTCATT TAATCTAAGC GACCACGCGC
 2501 TACAAAACGG CAGTCTGACG CTTTCGGGCA ACGCTAAGGC AAACGTAAGC
 2551 CATTCGCGAC TCAACGGTAA TGTCTCCCTA GCCGATAAGG CAGTATTCCA
 2601 TTTTGAAAGC AGCCGCTTTA CCGGACAAAT CAGCGGCGGC AAGGATACGG
 2651 CATTACACTT AAAAGACAGC GAATGGACGC TGCCGTCAGG CACGGAATTA
 2701 GGCAATTTAA ACCTTGACAA CGCCACCATT ACACTCAATT CCGCTATCG
 2751 CCACGATGCG GCAGGGGCGC AAACCGGCG TCGACAGAT GCGCCGCGCC
 2801 GCCGTCGCG CCGTTCGCGC CGTTCCCTAT TATCCGTTAC ACCGCCAACT
 2851 TCGGTAGAAAT CCCGTTTCAA CACGCTGACG GTAAACGGCA AATTGAACGG
 2901 TCAGGGAACA TTCCGCTTTA TGTGGAACCT CTTCCGGCTAC CGCAGCGACA
 2951 AATTGAAGCT GGCGGAAAGT TCCGAAGGCA CTTACACCTT GCGGTCACAG
 3001 AATACCGGCA ACGAACCTGC AAGCCTCGAA CAATTGACGG TAGTGGAAGG
 3051 AAAAGACAAC AAACCGCTGT CCGAAAACCT TAATTTCACC CTGCAAAACG
 3101 AACACGTCGA TGCCGGCGCG TGGCGTTACC AACTCATCCG CAAAGACGGC
 3151 GAGTTCGCCG TGCATAATCC GGTCAAAGAA CAAGAGCTTT CCGACAAACT
 3201 CGGCAAGGCA GAAGCCAAAA AACAGGCGGA AAAAGACAAC GCGCAAGGCC
 3251 TTGACGCGCT GATTGCGGCC GGGCGCGATG CCGTCGAAAA GACAGAAAGC
 3301 GTTGCCGAAC CGGCCCGGCA GGCAGGCGGG GAAAATGTCT GCATTATGCA
 3351 GCGGAGGAA GAGAAAAAAC GGGTGCAGGC GGATAAAGAC ACCGCCTTGG
 3401 CGAAACAGCG CGAAGCGGAA ACCCGGCGCG CTACCACCGC CTTCCCCGCG
 3451 GCCCGCGCG CCCGCGGGA TTTGCGCAA CTGCAACCCC AACCGAGGCC
 3501 CCAACCGCAG CGCGACCTGA TCAGCCGTTA TGCCAATAGC GGTTTGAGTG
 3551 AATTTCGCG CACGCTCAAC AGCGTTTTCG CCGTACAGGA CGAATTAGAC
 3601 CGCGTATTTG CCGAAGACCG CCGCAACGCC GTTTGGACAA GCGGCATCCG
 3651 GGACACCAAA CACTACCGTT CGCAAGATTT CCGCGCTTAC CGCAACAAA

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3701 CCGACCTGCG CCAAATCGGT ATGCAGAAAA ACCTCGGCAG CGGGCGCGTC
3751 GGCATCCTGT TTTCGCACAA CCGGACCGAA AACACCTTCG ACGACGGCAT
3801 CGGCAACTCG GCACGGCTTG CCCACGGCGC CGTTTTCGGG CAATACGGCA
3851 TCGACAGGTT CTACATCGGC ATCAGCGCGG GCGCGGGTT TAGCAGCGGC
3901 AGCCTTTCAG ACGGCATCGG AGGCAAAATC CGCCGCCGCG TGCTGCATTA
3951 CGGCATTGAG GCACGATACC GCGCCGGTTT CGGCGGATTG GGCATCGAAC
4001 CGCACATCGG CGCAACGCGC TATTTCGTCC AAAAAGCGGA TTACCGCTAC
4051 GAAAACGTCA ATATCGCCAC CCCCGGCCTT GCATTCAACC GCTACCGCGC
4101 GGGCATTAAAG GCAGATTATT CATTCAAACC GCGCAACAC ATTTCCATCA
4151 CGCCTTATTT GAGCCTGTCC TATACCGATG CCGCTTCGGG CAAAGTCCGA
4201 ACACGCGTCA ATACCGCCGT ATTGGCTCAG GATTTCGGCA AAACCCGCGA
4251 TCGGGAATGG GCGGTAAACG CCGAAATCAA AGTTTCACG CTGTCCCTCC
4301 ACGTGCCGCG CGCCAAAGGC CCGCAACTGG AAGCGCAACA CAGCGCGGGC
4351 ATCAAATTAG GCTACCGCTG GTAA

```

This corresponds to the amino acid sequence <SEQ ID 87; ORF orf1-1>:

orf1-1.pap

```

1 MKTTDKRTE THRKAPKTGR IRFSPAYLAI CLSFGILPQA WAGHTYFGIN
51 YQYYRDEFAEN KGKFAVGAKD IEVYNKKGEL VGKSMKAPM IDFSVVSRRNG
101 VAALVGDOYI VSVAHNGGYN NVDFGAEGRN PDQHRFTYKI VKRNNYKAGT
151 KGHPYGGDYH MPRLHKFVTD AEPVEMTSYM DGRKYIDQNN YPDRVRIGAG
201 RQYWRSEDEDE PNNRESSYHI ASAYSWLVGG NTFQNGSGG GTVNLGSEKI
251 KHSPYGFLEPT GGSFGDSGSP MFIYDAQKQK WLVINGVLQTF NPYIGKSNFG
301 QLVVRKDWFYD EIFAGDTHSV FYEPRONGKY SFNDDNNGTG KINAKHEHNS
351 LPNRLKTRTV QLFNVSLSET AREPVYHAAG GVNSYRPRLN NGENISFIDE
401 KGELILTSN INQAGGLYF QGDFTVSPEN NETWQAGGVH ISEDSTVTWK
451 VNGVANDRLS KIGKGTLVHQ AKGENQGSIS VGDGTVILDQ QADDKGGKQA
501 FSEIGLVSGR GTVQLNADNQ FNPDKLYFGF RGGRLDLNGH SLSFHRIGNT
551 DEGAMIVNHN QDKESTVTIT GNKDIAATGN NNSLDSKKEI AYNGWFGEKD
601 TTKTNGRLNL VYQPAEDRT LLLSGGTNLN GNITQTNGKL FFSGRPTPHA
651 YNHLNDHWSQ KEGIPRGEIV WDNDWINRTF KAENFQIKGG QAVVSRNVAK
701 VKGDWHLNSH AQAVFGVAPH QSHTICTRSD WTGLTNCVEK TITDDKVIAS
751 LTKTDISGNV DLADHAHLNL TGLATLNGNL SANGDTRYTV SHNATQNGNL
801 SLVGNAQATF NQATLNGNTS ASGNASFNLS DHAVQNGSLT LSGNAKANVS
851 HSLNGNVSL ADKAVFHFES SRFTGQISGG KDTALHLKDS EWTLPSTEL
901 GNLNLDNATI TLNSAYRHDA AGAQTGSATD APRRRSRRSR RSLSVTPPT
951 SVESRENTLT VNGKLNQGT FRFMSELFY RSDKLKLAES SEGTYTLAVN
1001 NTGNEPASLE QLTVEGKDN KPLSENLFNT LQNEHVDAGA WRYQLIRKDG
1051 EFRLHNPVKE QELSDKLGA EAKKQAEKDN AQSLDALIAA GRDAVEKTES
1101 VAEPARQAGG ENVGIMQAE EKKRVQADKD TALAKQREAE TRPATTAFPR
1151 ARRARRDLPO LQPQPQPQPQ RDLISRYANS GLSEFSATLN SVFAVQDELD
1201 RVFAEDRRNA VWTSGIRDTK HYRSQDFRAY RQQTDLRQIG MQKNLGSGRV
1251 GILFSHNRTE NTFDDGIGNS ARLAHGAVFG QYGIDRFYIG ISAGAGFSSG
1301 SLSDGIGGKI RRRVLHYGIQ ARYRAGFGGF GIEPHIGATR YFVQKADYRY
1351 ENVNIATPGL AFNRYRAGIK ADYSFKPAQH ISITPYLSLS YTDAASGKVR
1401 TRVNTAVLAQ DFGKTRSAEW GVNAEIKGFT LSLHAAAAGK PQLEAQHSAG
1451 IKLGYRW*

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 88>:

orf46-2.seq

```

1 TTGGGCATTT CCCGCAAAAT ATCCCTTATT CTGTCCATAC TGGCAGTGTG
51 CCTGCCGATG CATGCACACG CCTCAGATTT GGCAAACGAT TCTTTTATCC
101 GGCAGGTTCT CGACCGTCAG CATTTCGAAC CCGACGGGAA ATACCACCTA
151 TTCCGCAGCA GGGGGGAAC TGGCCAGCGC AGCGGCCATA TCGGATTGGG
201 AAAAAACAAA AGCCATCAGT TGGGCAACCT GATGATTCAA CAGGCGGCCA
251 TTAAAGGAAA TATCGCTAC ATTGTCCGCT TTCCGATCA CGGGCACGAA
301 GTCCATTCCC CCTTCGACAA CCATGCCTCA CATTCCGATT CTGATGAAGC
351 CGGTAGTCCC GTTGACGGAT TTAGCCTTTA CCGCATCCAT TGGGACGGAT

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401  ACGAACACCA TCCCGCCGAC GGCTATGACG GGCCACAGGG CGGCGGCTAT
451  CCGGCTCCCA AAGGCGCGAG GGATATATAC AGCTACGACA TAAAGGCGT
501  TGCCCAAAAT ATCCGCCTCA ACCTGACCGA CAACCGCAGC ACCGGACAAAC
551  GGCTTGCCGA CCGTTTCCAC AATGCCGGTA GTATGCTGAC GCAAGGAGTA
601  GGCGACGGAT TCAAACGCGC CACCCGATAC AGCCCCGAGC TGGACAGATC
651  GGGCAATGCC GCCGAAGCCT TCAACGGCAC TGCAGATATC GTTAAAAACA
701  TCATCGGCGC GGCAGGAGAA ATTGTGCGCG CAGGCGATGC CGTGCAGGGC
751  ATAAGCGAAG GCTCAAACAT TGCTGTCATG CACGGCTTGG GTCTGCTTTC
801  CACCGAAAAC AAGATGGCGC GCATCAACGA TTTGGCAGAT ATGGCGCAAC
851  TCAAAGACTA TGCCGCAGCA GCCATCCGCG ATTGGGCAGT CCAAAACCCC
901  AATGCCGCAC AAGGCATAGA AGCCGTCAGC AATATCTTTA TGGCAGCCAT
951  CCCCATCAAA GGGATTGGAG CTGTTGCGGG AAAATACGGC TTGGGCGGCA
1001 TCACGGCACA TCCTATCAAG CGGTCGCAGA TGGGCGCGAT CGCATTGCCG
1051 AAAGGGAAAT CCGCCGTCAG CGACAATTTT GCCGATGCGG CATACGCCAA
1101 ATACCCGTCC CCTTACCATT CCCGAAATAT CCGTTCAAAC TTGGAGCAGC
1151 GTTACGGCAA AGAAAACATC ACCTCCTCAA CCGTGCCGCC GTCAAACGGC
1201 AAAAAATGTCA AACTGGCAGA CCAACGCCAC CCGAAGACAG GCGTACCGTT
1251 TGACGGTAAA GGGTTTCCGA ATTTTGAGAA GCACGTGAAA TATGATACGA
1301 AGCTCGATAT TCAAGAATTA TCGGGGGCGG GTATACCTAA GGCTAAGCCT
1351 GTGTTTGATG CGAAACCGAG ATGGGAGGTT GATAGGAAGC TTAATAAATT
1401 GACAACTCGT GAGCAGGTGG AGAAAAATGT TCAGGAAATA AGGAACGGTA
1451 ATATAAACAG TAACTTTAGC CAACATGCTC AACTAGAGAG GGAAATTAAT
1501 AAATAAAAT CTGCCGATGA AATTAATTTT GCAGATGGAA TGGGAAAATT
1551 TACCGATAGC ATGAATGACA AGGCTTTTAG TAGGCTTGTG AAATCAGTTA
1601 AAGAGAATGG CTTCACAAAT CCAGTTGTGG AGTACGTTGA AATAAATGGA
1651 AAAGCATATA TCGTAAGAGG AAATAATRGG GTTTTGTCTG CAGAATACCT
1701 TGGCAGGATA CATGAATTAA AATTTAAAAA AGTTGACTTT CCTGTTCCTA
1751 ATACTAGTTG GAAAAATCCT ACTGATGTCT TGAATGAATC AGGTAATGTT
1801 AAGAGACCTC GTTATAGGAG TAAATAA

```

This corresponds to the amino acid sequence <SEQ ID 89; ORF orf46-2>:

orf46-2.ppep

```

1  LGISRKISLI LSILAVCLPM HAHASDLAND SFIRQVLDRO HFEPDGKYHL
51  FGSRGELAER SGHIGLGKIQ SHQLGNLMIQ QAAIKGNIGY IVRFSDHGHE
101 VHSPPFDNHAS HSDSDEAGSP VDGFSLYRIH WDGYEHHPAD GYDGPQGGGY
151 PAPKGARDIY SYDIKGVAQN IRLNLTDNRS TGQRLADRFH NAGSMLTQGV
201 GDGFKRATRY SPELDRSGNA AEAFTGTADI VKNIIGAAGE IVGAGDAVQG
251 ISEGSNIAVM HGLGLLSTEN KMARINDLAD MAQLKDYAAA AIRDWAVQNP
301 NAAQGIEAVS NIFMAAIIPIK GIGAVRGKYG LGGITAHPIK RSQMGAIALP
351 KGKSAVSDNF ADAAYAKYPS PYHSRNIRSN LEQRYGKENI TSSTVPPSNG
401 KNVKLADQRH PKTGVPFDGK GFPNFEKHVK YDTKLDIQEL SGGGIPKAKP
451 VFDAPRWEV DRKLNKLTTT EQVEKNVQEI RNGNINSNFS QHAQLEREIN
501 KLKSADEINF ADGMGKFTDS MNDKAFSRLV KSVKENGFTN PVVEYVEING
551 KAYIVRGNNR VFAAEYLGRI HELKFKKVDV PVPNTSWKNP TDVLNESGNV
601 KRPRYRSK*

```

Using the above-described procedures, the following oligonucleotide primers were employed in the polymerase chain reaction (PCR) assay in order to clone the ORFs as indicated:

Oligonucleotides used for PCR

Table 1

The primer described in Table 1 for ORF 919 was used to locate and clone ORF 919. The predicted gene *919* was cloned in pET vector and expressed in *E. coli*. The product of

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protein expression and purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 919-His fusion protein purification. Mice were immunized with the purified 919-His and sera were used for Western blot (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Symbols: M1, molecular weight marker; PP, purified protein, TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 919 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 919 are provided in Figure 10. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 919 and the amino acid sequence encoded thereby is provided in Example 1.

EXAMPLE 3

Expression of ORF 279

The primer described in Table 1 for ORF 279 was used to locate and clone ORF 279. The predicted gene 279 was cloned in pGex vector and expressed in *E. coli*. The product of protein expression and purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 279-GST purification. Mice were immunized with the purified 279-GST and sera were used for Western blot analysis (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 279 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 279 are provided in Figure 11. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 279 and the amino acid sequence encoded thereby is provided in Example 1.

The primer described in Table 1 for ORF 519 was used to locate and clone ORF 519. The predicted gene 519 was cloned in pET vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 519-His fusion protein purification. Mice were immunized with the purified 519-His and sera were used for Western blot (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 519 is a surface-exposed protein

- 119 -

and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 519 are provided in Figure 13. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 519 and the amino acid sequence encoded thereby is provided in Example 1.

EXAMPLE 6

Expression of ORF 121

The primer described in Table 1 for ORF 121 was used to locate and clone ORF 121. The predicted gene *121* was cloned in pET vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 121-His fusion protein purification. Mice were immunized with the purified 121-His and sera were used for Western blot analysis (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Results show that 121 is a surface-exposed protein. Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 121 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 121 are provided in Figure 14. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 121 and the amino acid sequence encoded thereby is provided in Example 1.

EXAMPLE 7

Expression of ORF 128

The primer described in Table 1 for ORF 128 was used to locate and clone ORF 128. The predicted gene *128* was cloned in pET vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 128-His purification. Mice were immunized with the purified 128-His and sera were used for

- 120 -

Western blot analysis (panel B), FACS analysis (panel C), bactericidal assay (panel D) and ELISA assay (panel E). Results show that 128 is a surface-exposed protein. Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 128 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 128 are provided in Figure 15. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 128 and the amino acid sequence encoded thereby is provided in Example 1.

EXAMPLE 8

Expression of ORF 206

The primer described in Table 1 for ORF 206 was used to locate and clone ORF 206. The predicted gene 206 was cloned in pET vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 206-His purification. Mice were immunized with the purified 206-His and sera were used for Western blot analysis (panel B). It is worthnoting that the immunoreactive band in protein extracts from meningococcus is 38 kDa instead of 17 kDa (panel A). To gain information on the nature of this antibody staining we expressed ORF 206 in *E. coli* without the His-tag and including the predicted leader peptide. Western blot analysis on total protein extracts from *E. coli* expressing this native form of the 206 protein showed a recative band at a position of 38 kDa, as observed in meningococcus. We conclude that the 38 kDa band in panel B) is specific and that anti-206 antibodies, likely recognize a multimeric protein complex. In panel C is shown the FACS analysis, in panel D the bactericidal assay, and in panel E) the ELISA assay. Results show that 206 is a surface-exposed protein. Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 206 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots,

- 121 -

antigenic index, and amphipatic regions of ORF 519 are provided in Figure 16. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 206 and the amino acid sequence encoded thereby is provided in Example 1.

EXAMPLE 9

Expression of ORF 287

The primer described in Table 1 for ORF 287 was used to locate and clone ORF 287. The predicted gene 287 was cloned in pGex vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 287-GST fusion protein purification. Mice were immunized with the purified 287-GST and sera were used for FACS analysis (panel B), bactericidal assay (panel C), and ELISA assay (panel D). Results show that 287 is a surface-exposed protein. Symbols: M1, molecular weight marker. Arrow indicates the position of the main recombinant protein product (A). These experiments confirm that 287 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 287 are provided in Figure 17. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 287 and the amino acid sequence encoded thereby is provided in Example 1.

EXAMPLE 10

Expression of ORF 406

The primer described in Table 1 for ORF 406 was used to locate and clone ORF 406. The predicted gene 406 was cloned in pET vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 406-His fusion protein purification. Mice were immunized with the purified 406-His and sera were used for Western blot analysis (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Results show that 406 is a surface-exposed protein. Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N.*

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meningitidis outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 406 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipathic regions of ORF 406 are provided in Figure 18. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 406 and the amino acid sequence encoded thereby is provided in Example I.

The foregoing examples are intended to illustrate but not to limit the invention.

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Claims

1. A method for identifying an amino acid sequence, comprising the step of searching for putative open reading frames or protein-coding sequences within one or more of *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.
2. A method according to claim 1, comprising the steps of searching a *N. meningitidis* nucleotide sequence for an initiation codon and searching the upstream sequence for an in-frame termination codon.
3. A method for producing a protein, comprising the step of expressing a protein comprising an amino acid sequence identified according to any one of claims 1-2.
4. A method for identifying a protein in *N. meningitidis*, comprising the steps of producing a protein according to claim 3, producing an antibody which binds to the protein, and determining whether the antibody recognises a protein produced by *N. meningitidis*.
5. Nucleic acid comprising an open reading frame or protein-coding sequence identified by a method according to any one of claims 1-2.
6. A protein obtained by the method of claim 3.
7. Nucleic acid comprising one or more of the *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.
8. Nucleic acid comprising a nucleotide sequence having greater than 50% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.

- 124 -

9. Nucleic acid comprising a fragment of a nucleotide sequence selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.
10. Nucleic acid according to claim 9, wherein the fragment is unique to the genome of *N. meningitidis*.
11. Nucleic acid complementary to the nucleic acid of any one of claims 7-10.
12. A protein comprising an amino acid sequence encoded within one or more of the *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.
13. A protein comprising an amino acid sequences having greater than 50% sequence identity to an amino acid sequence encoded within one or more of the *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.
14. A protein comprising a fragment of an amino acid sequence encoded within one or more of the *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.
15. Nucleic acid encoding a protein according to any one of claims 6-8.
16. A computer, a computer memory, a computer storage medium or a computer database containing the nucleotide sequence of a nucleic acid according to any one of claims 7-11.
17. A computer, a computer memory, a computer storage medium or a computer database containing one or more of the *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.

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18. A polyclonal or monoclonal antibody which binds to a protein according to any one of claims 12-14 or 6.

19. A nucleic acid probe comprising nucleic acid according to any one of claims 5, 7-10, or 15.

20. An amplification primer comprising nucleic acid according to any one of claims 5, 7-10, or 15.

21. A composition comprising (a) nucleic acid according to any one of claims 5, 7-10, or 15; (b) protein according to any one of claims 12-14; and/or (c) an antibody according to claim 18.

22. The use of a composition according to claim 21 as a medicament or as a diagnostic reagent.

23. The use of a composition according to claim 21 in the manufacture of (a) a medicament for treating or preventing infection due to Neisserial bacteria and/or (b) a diagnostic reagent for detecting the presence of Neisserial bacteria or of antibodies raised against Neisserial bacteria.

24. A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 21.



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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C12Q 1/68, C12N 15/11, C07K 14/22		A1	(11) International Publication Number: WO 00/66791
			(43) International Publication Date: 9 November 2000 (09.11.00)
(21) International Application Number: PCT/US00/05928		<p>[IT/IT]: Chiron SpA, Via Fiorentina 1, I-53100 Siena (IT). MORA, Marirosa [IT/IT]; Chiron SpA, Via Fiorentina, I, I-53100 Siena (IT). RATTI, Giulio [IT/IT]; Chiron SpA, Via Fiorentina, I, I-53100 Siena (IT). SCARSELLI, Maria [IT/IT]; Chiron SpA, Via Fiorentina, I, I-53100 Siena (IT). SCARLATO, Vincenzo [IT/IT]; Chiron SpA, Via Fiorentina, I, I-53100 Siena (IT). RAPPUOLI, Rino [IT/IT]; Chiron SpA, Via Fiorentina, I, I-53100 Siena (IT). FRAZER, Claire, M. [US/US]; 4560 Horton Street, Emeryville, CA 94608 (US). GRANDI, Guido [IT/IT]; Chiron SpA, Via Fiorentina, I, I-53100 Siena (IT).</p> <p>(74) Agent: HARBIN, Alisa, A.; Chiron Corporation, Intellectual Property - R440, P.O. Box 8097, Emeryville, CA 94662-8097 (US).</p> <p>(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p>	
(22) International Filing Date: 8 March 2000 (08.03.00)			
(30) Priority Data:			
60/132,068 30 April 1999 (30.04.99) US PCT/US99/23573 8 October 1999 (08.10.99) US 0004695.3 28 February 2000 (28.02.00) GB			
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		<p>Published</p> <p>With international search report.</p> <p>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</p>	

(54) Title: NEISSERIA GENOMIC SEQUENCES AND METHODS OF THEIR USE

A

919 (46 kDa)

PURIFICATION

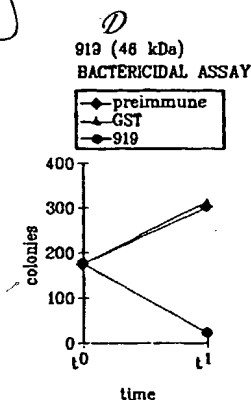
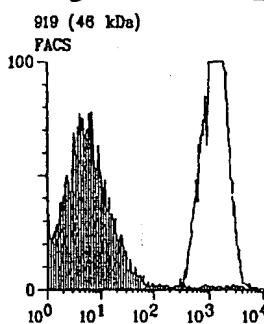
M1 919

B

919 (46 kDa)

WESTERN BLOT

OMV TP PP



E

919 (46 kDa)

ELISA assay: positive

(57) Abstract

The invention provides methods of obtaining immunogenic proteins from genomic sequences including *Neisseria*, including the amino acid sequences and the corresponding nucleotide sequences, as well as the genomic sequence of *Neisseria meningitidis B*. The proteins so obtained are useful antigens for vaccines, immunogenic compositions, and/or diagnostics.

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DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

FIG. 1A

919 (46 kDa)

PURIFICATION

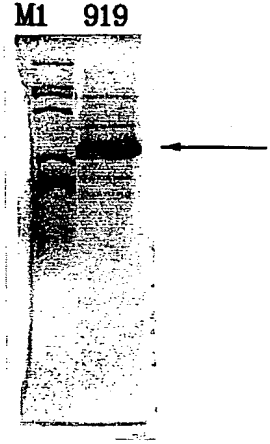


FIG. 1B

919 (46 kDa)

WESTERN BLOT
OMV TP PP

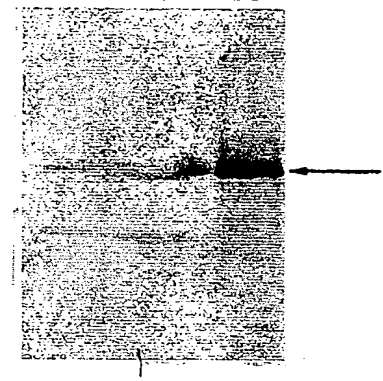


FIG. 1C

919 (46 kDa)

FACS

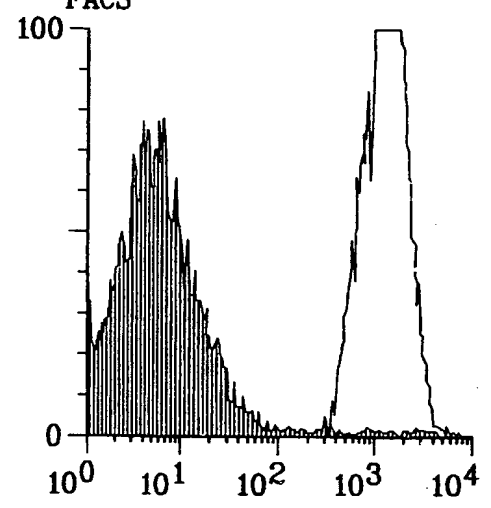


FIG. 1D

919 (46 kDa)

BACTERICIDAL ASSAY

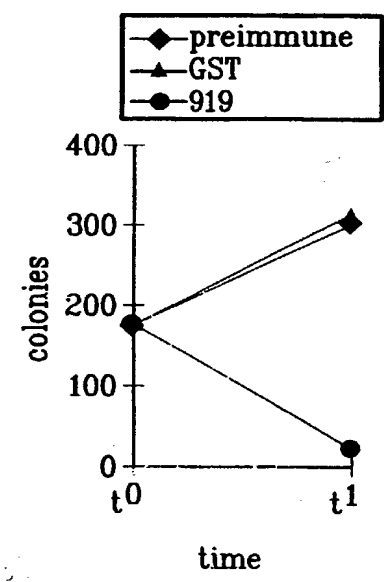


FIG. 1E

919 (46 kDa)

ELISA assay: positive

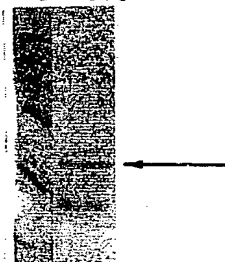
2/18

FIG. 2A

279 (10.5 kDa)

PURIFICATION

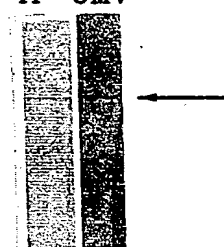
M1 279

*FIG. 2B*

279 (10.5 kDa)

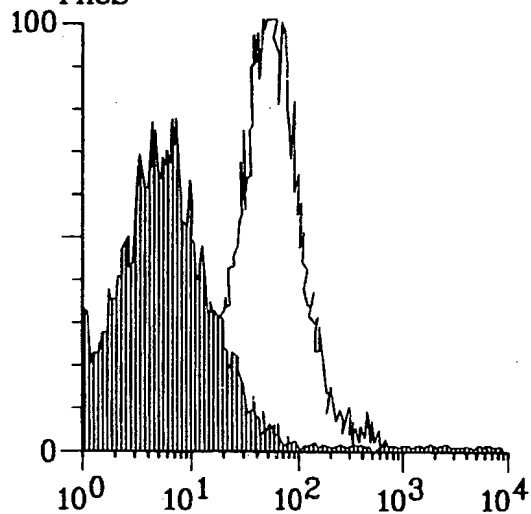
WESTERN BLOT

TP OMV

*FIG. 2C*

279 (10.5 kDa)

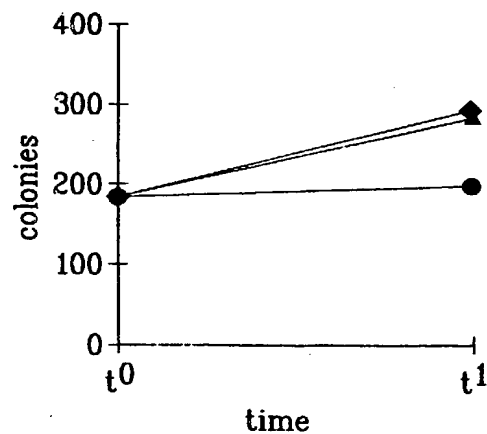
FACS

*FIG. 2D*

279 (10.5 kDa)

BACTERICIDAL ASSAY

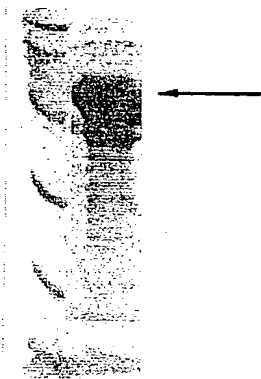
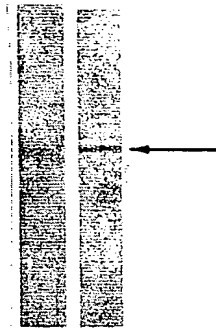
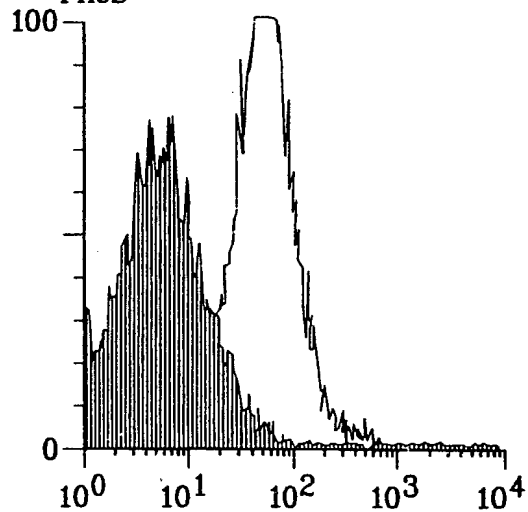
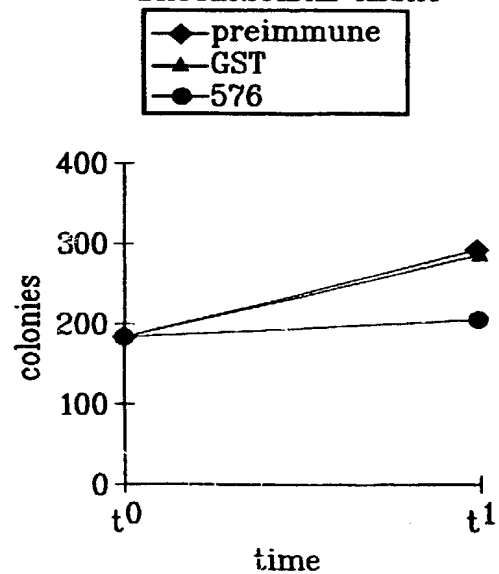
◆ preimmune
▲ GST
● 279

*FIG. 2E*

279 (10.5 kDa)

ELISA assay: positive

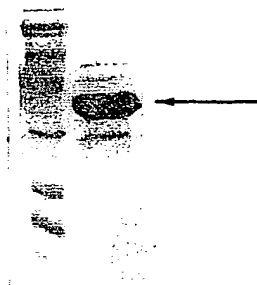
3/18

*FIG. 3A*576 (27.8 kDa)
PURIFICATION
M1 576*FIG. 3B*576 (27.8 kDa)
WESTERN BLOT
TP OMV*FIG. 3C*576 (27.8 kDa)
FACS*FIG. 3D*576 (27.8 kDa)
BACTERICIDAL ASSAY*FIG. 3E*576 (27.8 kDa)
ELISA assay: positive

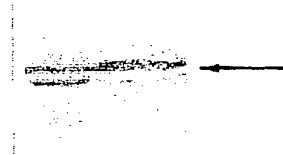
4/18

FIG. 4A

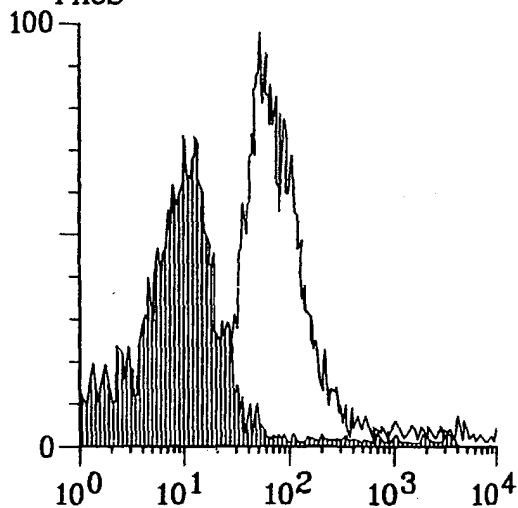
519 (33 kDa)
PURIFICATION
M1 519

*FIG. 4B*

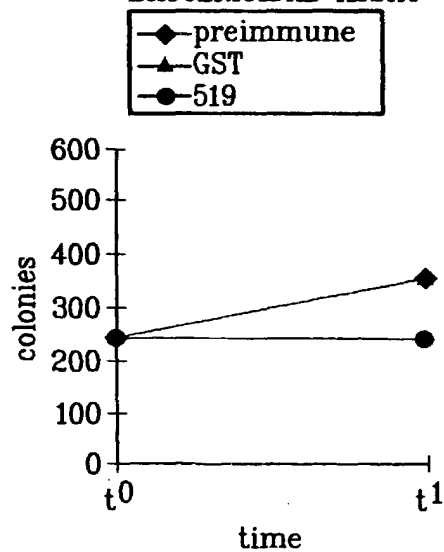
519 (33 kDa)
WESTERN BLOT
TP OMV

*FIG. 4C*

519 (33 kDa)
FACS

*FIG. 4D*

519 (33 kDa)
BACTERICIDAL ASSAY

*FIG. 4E*

519 (33 kDa)
ELISA assay: positive

5/18

FIG. 5A

121 (40 kDa)
PURIFICATION
M1 121



FIG. 5B

121 (40 kDa)
WESTERN BLOT
TP OMV

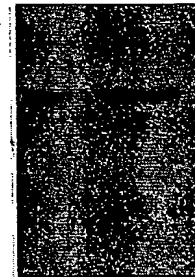


FIG. 5C

121 (40 kDa)
FACS

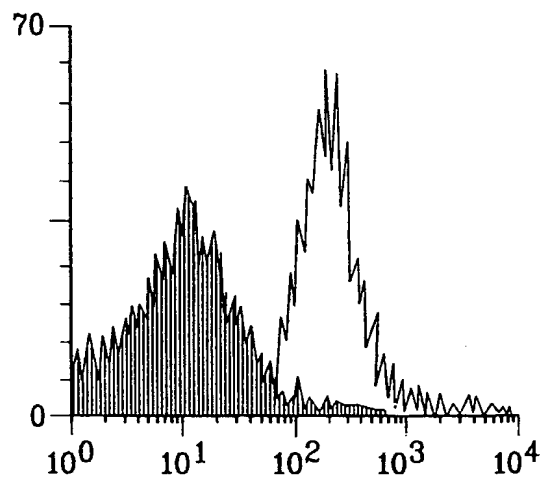


FIG. 5D

121 (40 kDa)
BACTERICIDAL ASSAY

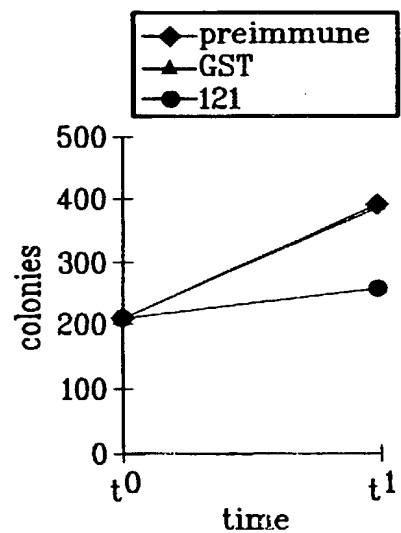


FIG. 5E

121 (40 kDa)

ELISA assay: positive

6/18

FIG. 6A

128 (101 kDa)

PURIFICATION

M1 128

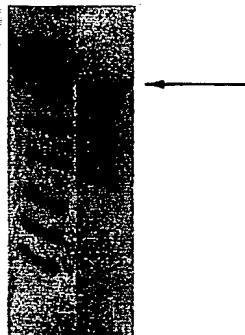


FIG. 6B

128 (101 kDa)

WESTERN BLOT

TP OMV

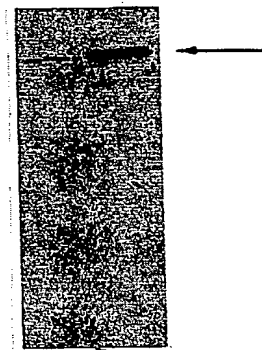


FIG. 6C

128 (101 kDa)

FACS

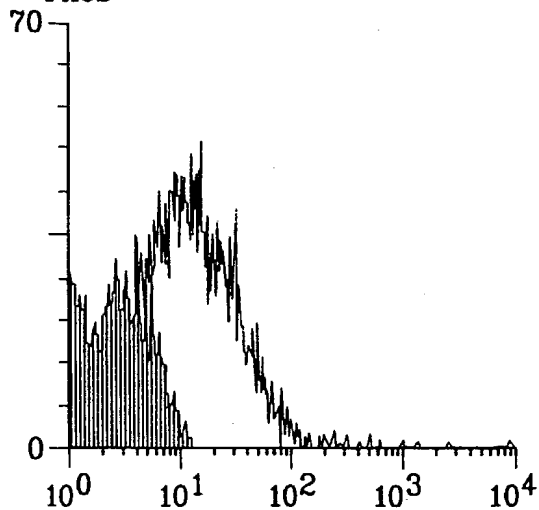


FIG. 6D

128 (101 kDa)

BACTERICIDAL ASSAY

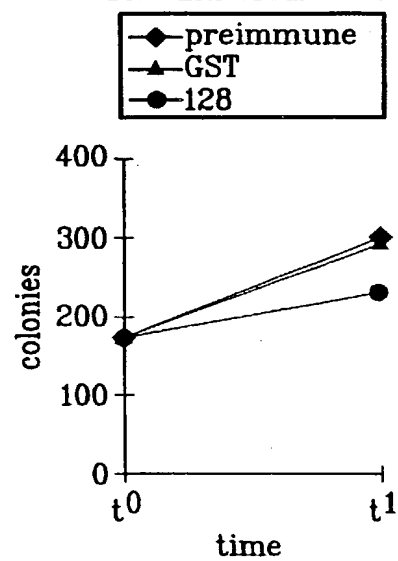
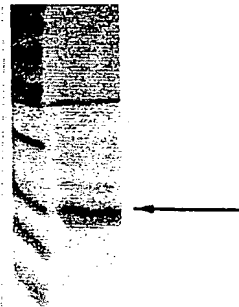
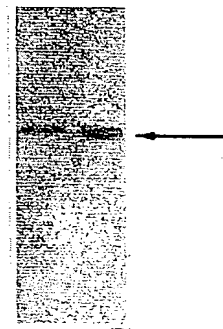
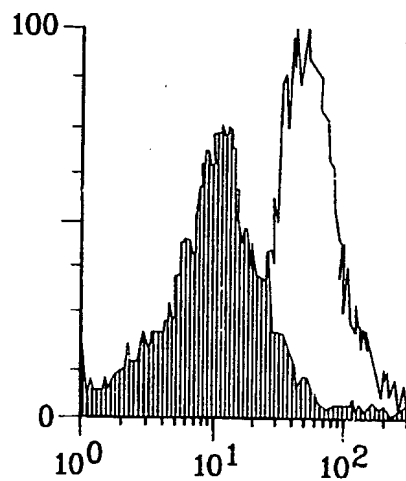
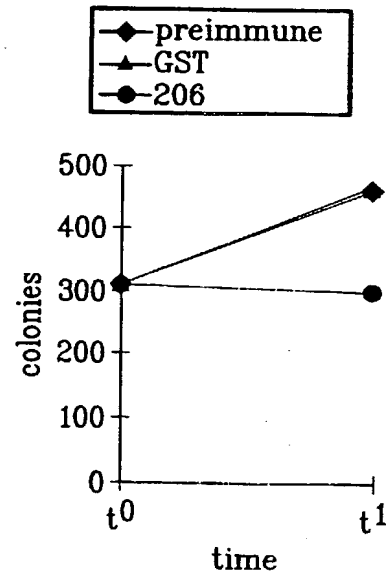


FIG. 6E

128 (101 kDa)

ELISA assay: positive

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*FIG. 7A*206 (17 kDa)
PURIFICATION
M1 206*FIG. 7B*206 (17 kDa)
WESTERN BLOT
TP OMV*FIG. 7C*206 (17 kDa)
FACS*FIG. 7D*206 (17 kDa)
BACTERICIDAL ASSAY*FIG. 7E*206 (17 kDa)
ELISA assay: positive

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FIG. 8A

287 (78 kDa)

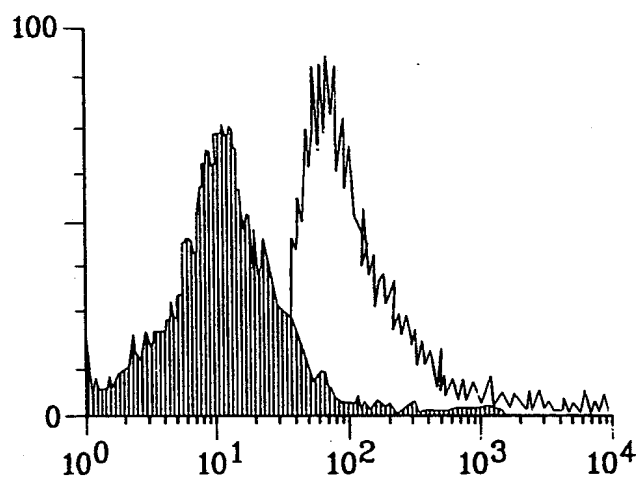
PURIFICATION

M1 287

*FIG. 8B*

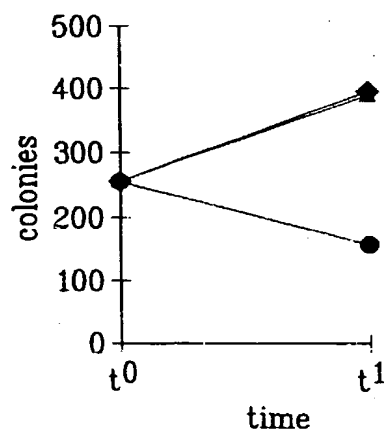
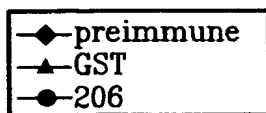
287 (78 kDa)

FACS

*FIG. 8C*

287 (78 kDa)

BACTERICIDAL ASSAY

*FIG. 8D*

287 (78 kDa)

ELISA assay: positive

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FIG. 9A
406 (33 kDa)
PURIFICATION
M1 406

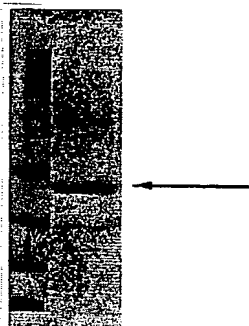


FIG. 9B
406 (33 kDa)
WESTERN BLOT
TP OMV

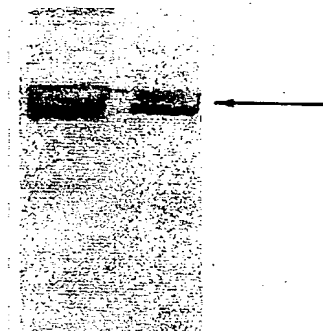


FIG. 9C
406 (33 kDa)
FACS

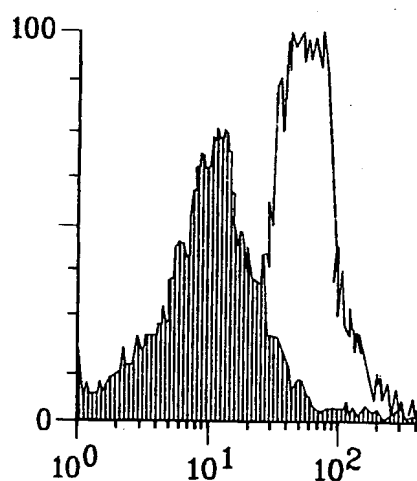


FIG. 9D
406 (33 kDa)
BACTERICIDAL ASSAY

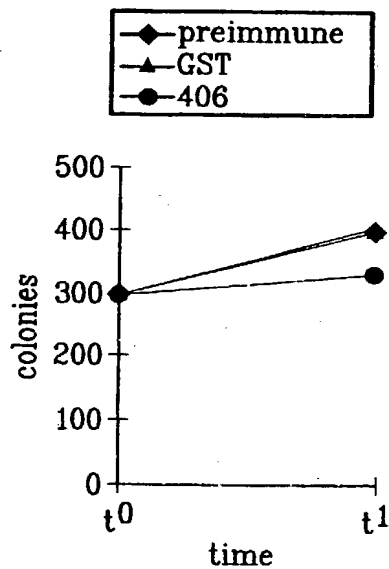


FIG. 9E
406 (33 kDa)
ELISA assay: positive

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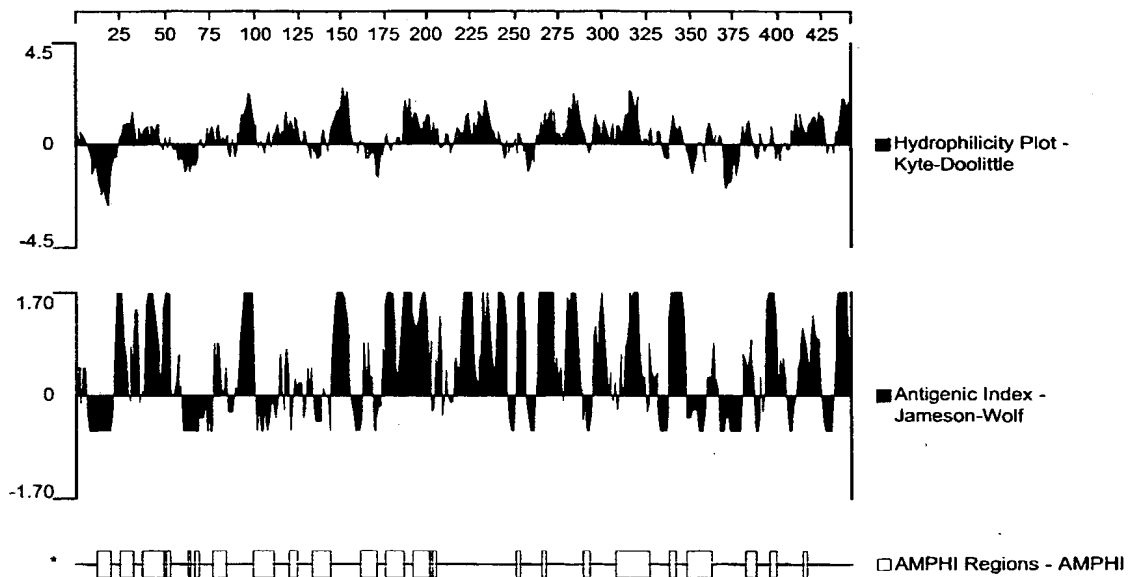
Hydrophilicity Plot, Antigenic Index and AMPHI Regions

Fig. 10

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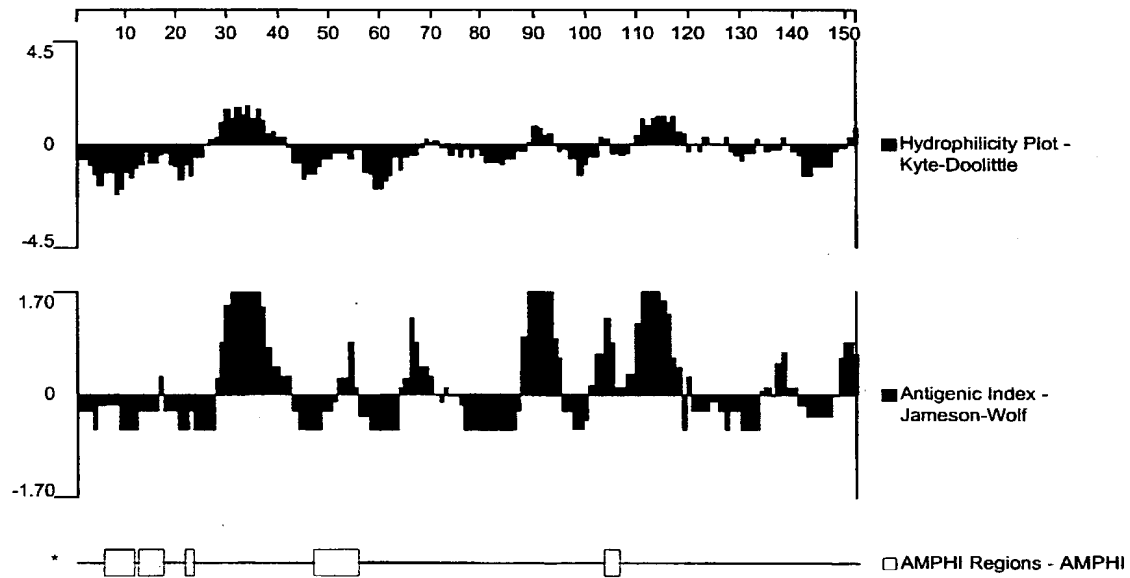
279Hydrophilicity Plot, Antigenic Index and AMPHI Regions

Fig. 11

WO 00/66791

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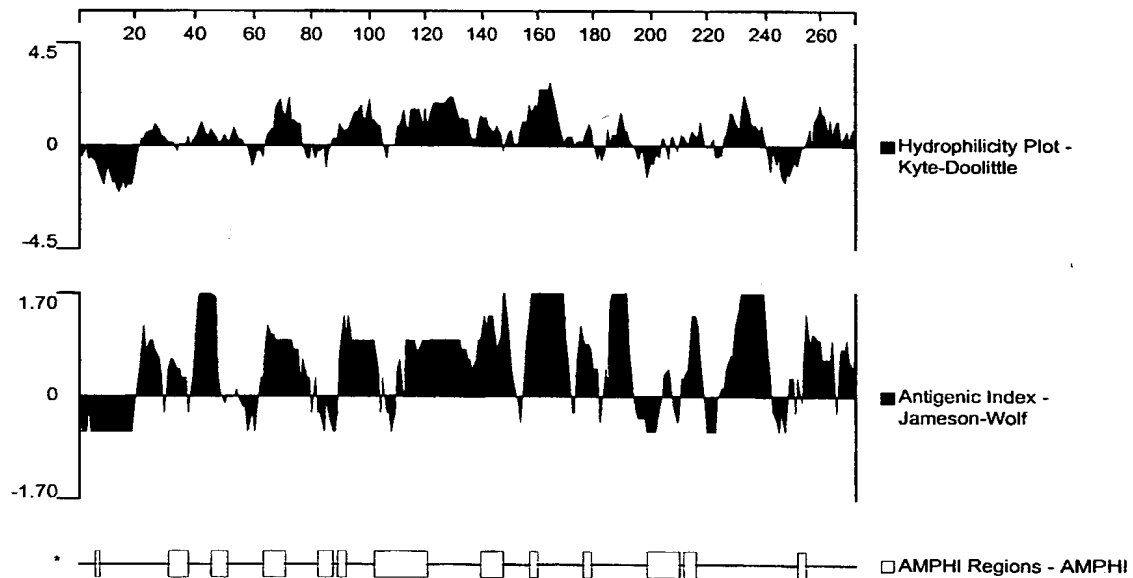
576-1Hydrophilicity Plot, Antigenic Index and AMPHI Regions

Fig. 12

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519-1
Hydrophilicity Plot, Antigenic Index and AMPHI Regions

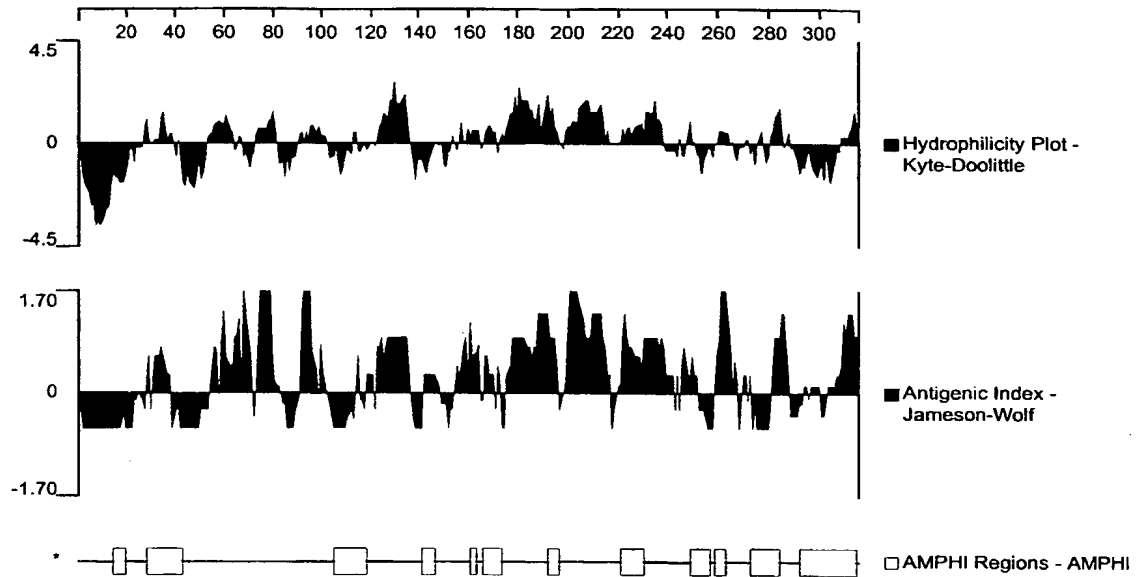


Fig. 13

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121-1
Hydrophilicity Plot, Antigenic Index and AMPHI Regions

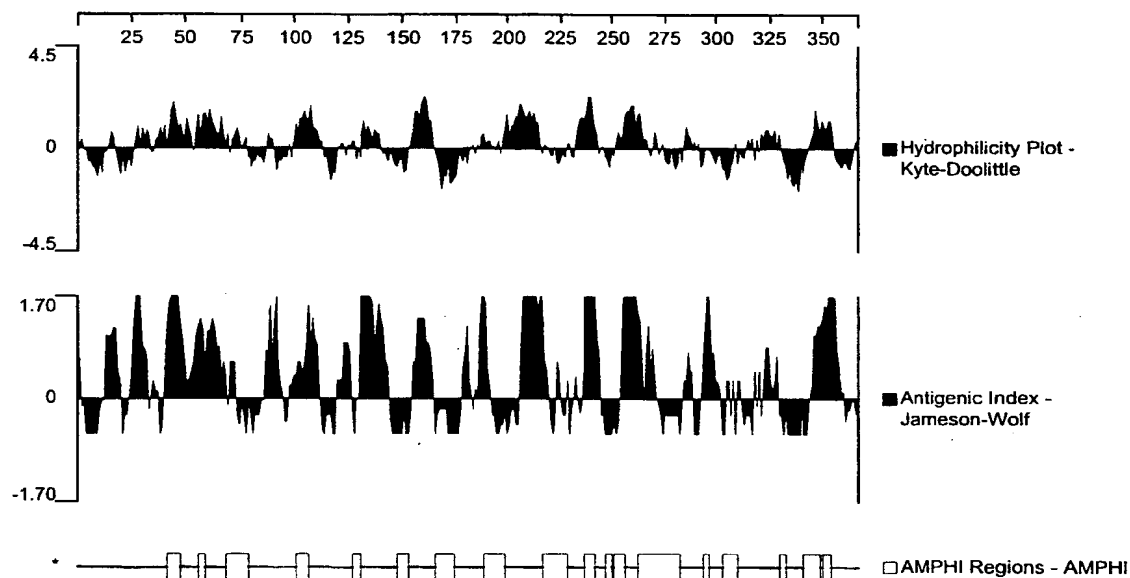


Fig. 14

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128-1
Hydrophilicity Plot, Antigenic Index and AMPHI Regions

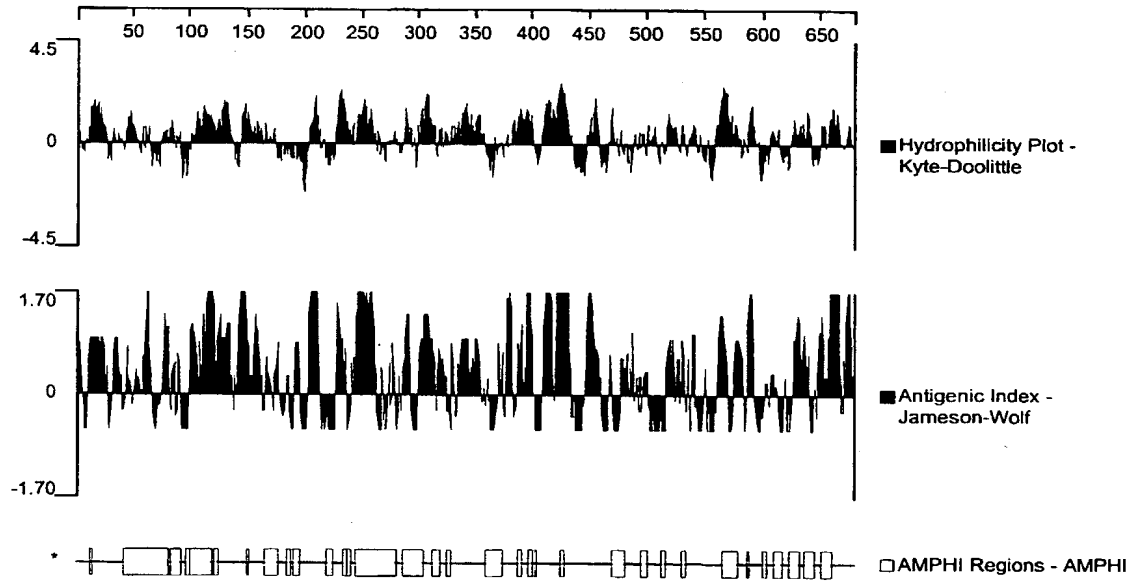


Fig. 15

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Hydrophilicity Plot, Antigenic Index and AMPHI Regions

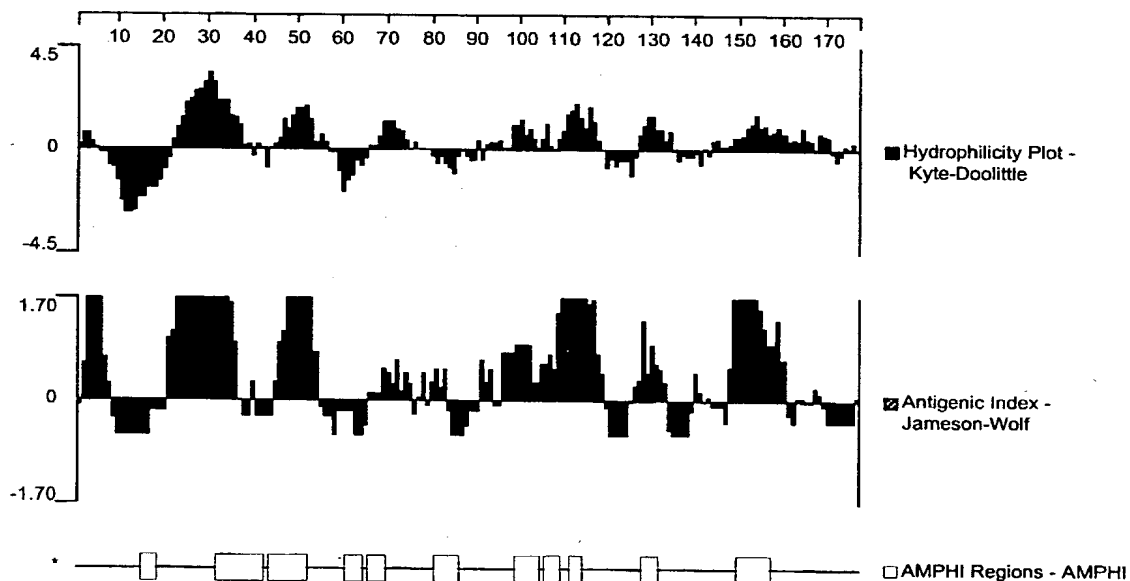


Fig. 16

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Hydrophilicity Plot, Antigenic Index and AMPHI Regions

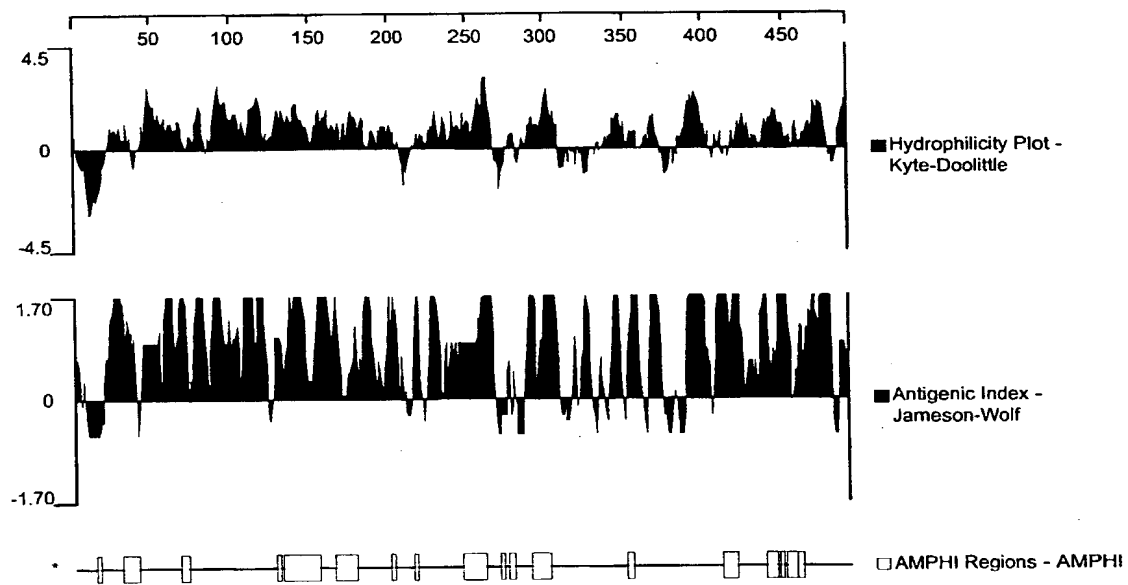


Fig. 17

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406

Hydrophilicity Plot, Antigenic Index and AMPHI Regions

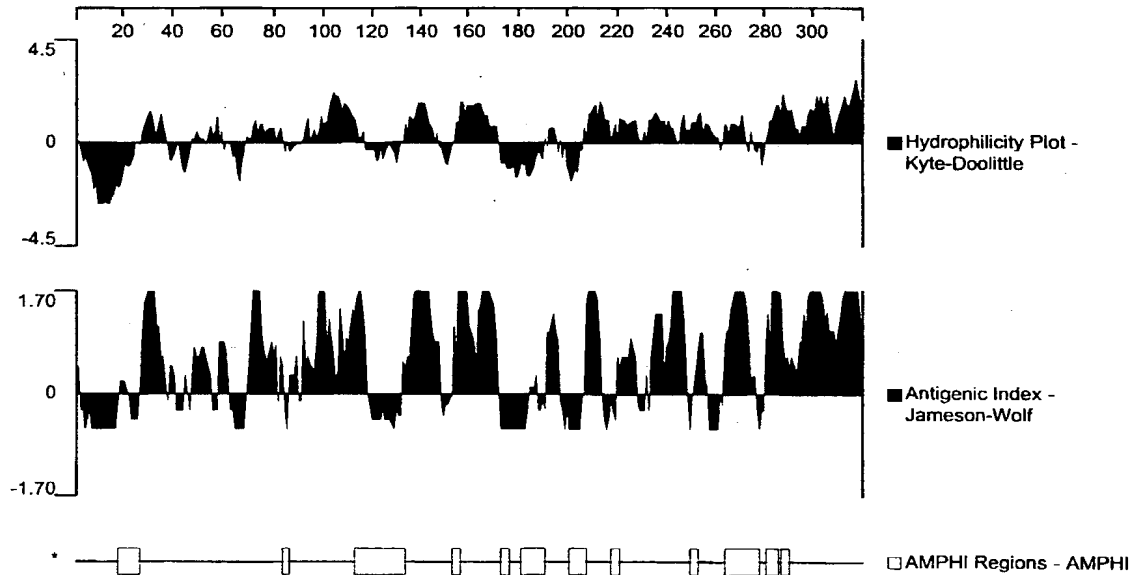


Fig. 18

DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **NEISSERIA GENOMIC SEQUENCES AND METHODS OF THEIR USE**

the specification of which (check one) is attached hereto x was filed on March 8, 2000, as International Application No. PCT/US00/05928, and was amended on (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
Number	Country	Day/Month/Year Filed	Yes	No
US00/05928	PCT	March 8, 2000	X	
0004695.3	GB	Feb. 28, 2000	X	
US99/23573	PCT	Oct. 8, 1999	X	

I hereby claim the benefit under Title 35, United States Code, §120 and/or §119(e) of any United States application(s) and/or provisional applications listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status Patented, Pending, Abandoned
60/132,068	April 30, 1999	Abandoned

101 Rec'd PCT/PTO 21 NOV 2002

PATENT

Atty Dkt. No. PP00365.322

#4

I hereby certify that this paper is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" on **November 21, 2002** and addressed to: BOX PCT, Assistant Commissioner of Patent and Trademarks, Washington, D.C. 20231.

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Gyne Riser

11.21.02
Date

10/018470

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of: **Pizza, M., et al.**

U.S. Serial No.: **10/018,470**

Intl. Appln. No.: **PCT/US00/05928**

Group Art Unit: **not yet assigned**

Int. Filing Date: **March 8, 2000**

Examiner: **not yet assigned**

For: **NEISSERIA GENOMIC SEQUENCES AND METHODS OF THEIR USE**

POWER OF ATTORNEY

Assistant Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

Pursuant to 37 C.F.R. §1.36, Chiron Corporation, the Assignee of the above-identified patent application hereby appoints the following attorneys and agents to prosecute this application and to transact all business in the Patent and Trademark Office in connection therewith.

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Dated: 11/21/02

CHIRON CORPORATION
By: Alisa A. Harbin
Assistant Secretary

4

I hereby declare that all statements herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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PATENT
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Atty. Docket No. PP00365.322

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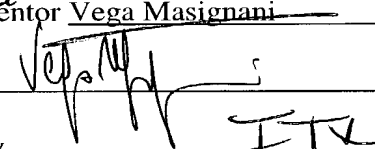
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Full name of eighth inventor Marirosa Mora

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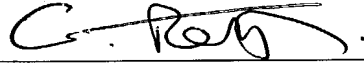
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Atty. Docket No. PP00365.322

8-00

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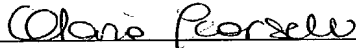
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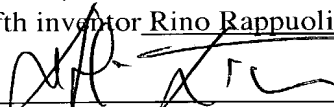
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Intl. App. No. PCT/US00/05928

Atty. Docket No. PP00365.322

12-00
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<110> Frazer, Claire M.
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 Pizza, Mariagratia
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$\frac{1}{\sqrt{\pi}} \int_{-\infty}^{\infty} f(x) \delta(x-a) dx = f(a)$

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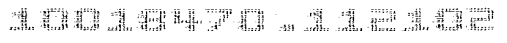
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(The following information was obtained from the records of the Department of Social Services, State of New York.)

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